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ANTIGENIC AND PROTECTIVE PROPERTIES OF EXPERIMENTAL ASSOCIATED VIRUS VACCINE AGAINST SHEEP POX AND GOAT POX

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SUMMARY

Studies have been carried out on antigenic and protective activity of the experimental associated vaccine containing, in the field vaccination dose, antigens of the attenuated ARRIAH strain of the sheep pox virus and ARRIAH 2003 strain of the goat pox virus in the ratio of 4.24 and 4.24 lg TCD_{50}/cm^3, 4.18 and 4.37 lg TCD_{50}/cm^3 and 4.37 and 4.18 lg TCD_{50}/cm^3 respectively, as well as monovalent vaccines with the infectious activity of these strains with a titer of 4.5 lg TCD_{50}/cm^3. Administration of the associated virus vaccine did not adversely affect the physiological state of the animals and was accompanied by the formation of viral neutralizing antibodies in protective titres that did not differ from antibody titers in the blood of sheep and goats during monovalent immunization. High levels of virus neutralizing antibodies in the range of $(3.50 \pm 0.50) – (4.05 \pm 0.22)$ and $(3.58 \pm 0.08) – (4.00 \pm 0.29)$ log_2 respectively were determined in the blood serum of all sheep and goats vaccinated with associated vaccines. The antigenic activity of the associated vaccines for goats and sheep is almost identical. There were no negative effects on the antigenic activity of different ratios of attenuated strains of the sheep pox and goat pox virus in the inoculation dose. All sheep immunized with the monovalent vaccine against sheep and goat pox and associated virus vaccines, resisted the disease when challenged with a virulent sheep pox virus "Afghanistan" strain. Three sheep vaccinated with a monovalent vaccine against goat pox demonstrated a large local reaction, one sheep was diagnosed with generalized form of sheep pox.

Key words: associated virus vaccine, antibodies, strains, sheep pox virus, goat pox, field vaccination dose.

INTRODUCTION

Sheep pox and goat pox are considered highly dangerous animal diseases. The causative agents are closely related but taxonomically independent strains of the sheep and goat pox virus belonging to the *Capripoxvirus* genus in the *Poxviridae* family and causing a severe disease form in one species only. According to the OIE disease classification sheep and goat pox is included in the category "Sheep and goat diseases" [9], and according to Order No. 476 of the Ministry of Agriculture of the Russian Federation of 09 December 2011 sheep and goat pox is included in the "List of contagious, including dangerous, animal diseases subject to restrictive measures (quarantine)".

The disease is characterized by lesions on the skin and mucous membranes, the formation of rash transitioning to papules and vesicles, and then to pustules on hairless skin areas, as well as serous-mucous or purulent discharge from the eyes and nose, increased body temperature. Saigas, goats, gazelles are susceptible to sheep pox. Lethality, depending on the breed, age of the animals and the form of the disease can reach 50–100% [2, 5].

Sheep and goat pox is a widespread disease. In 2016–2017 the outbreaks were reported in 34 countries of the African, Asian and European regions. The greatest disease threat for the Russian Federation comes from the following transboundary countries: China, Mongolia, Turkey,

Table 1
Study results of antigenic and protective activity of the associated virus vaccine against sheep and goat pox in sheep

Animal Group No.	Virus vaccine	SP и GP virus ratio in the field immunization dose (lg TCD_{50}/cm^3)	Post vaccination antibody titer (log_2), days			Protectivity	
			Against virus	14	21	Local reactions	generalization
1	SP and GP	4.24 + 4.24 (1:1)	SP	3.00 ± 0.21	3.63 ± 0.13	1/3*	–
			GP	3.27 ± 0.21	3.50 ± 0.50	–	–
2	SP and GP	4.18 + 4.37 (1:3)	SP	3.17 ± 0.31	4.00 ± 0.50	–	–
			GP	3.33 ± 0.31	3.51 ± 0.98	–	–
3	SP and GP	4.37 + 4.18 (3:1)	SP	3.33 ± 0.12	4.05 ± 0.22	–	–
			GP	3.25 ± 0.20	3.87 ± 0.12	–	–
4	SP	4.5	SP	3.62 ± 0.12	4.08 ± 0.31	–	–
			GP	2.50 ± 0.50	2.25 ± 0.25	–	–
5	GP	4.5	SP	2.75 ± 0.25	3.75 ± 0.25	–	–
			GP	3.12 ± 0.37	4.17 ± 0.23	3/3	1/3
6	control	–				2/2	2/2

* The numerator shows the number of positive sheep; the denominator shows the number of animals in the experiment.

where 88, 122 and 132 outbreaks respectively were detected during this period.

In 2010–2017 the territory of the Russian Federation (except for 2014 and 2017) was infected with sheep and goat pox [4]. The analysis of the epizootic situation in the world with regard to this disease indicates an exacerbation of the situation with the possibility of introduction of the infection into the territory of the Russian Federation, in particular, in the Siberian Federal District, the Zabaikalsky Krai, the Amur Oblast and the Khabarovsk Krai, the Chechen Republic, the Republic of Dagestan, the Primorsky Krai etc. [3, 4].

Given the high degree of danger of sheep pox and goat pox in the Russian Federation, it is necessary to carry out preventive vaccination in these regions using highly effective vaccines. In various countries monovalent vaccines are widely used providing reliable protection for animals against sheep pox and goat pox [2, 7].

A monovalent vaccine against sheep pox is often used for immunization of goats, however it does not protect animals from the disease. Similar results were obtained when immunizing sheep with a monovalent virus vaccine against goat pox [8]. With the threat of sheep pox and goat pox, the use of an associated virus vaccine is more promising, it allows to protect animals from the disease in the shortest time possible. There are reports of successful developments in this direction by I. L. Bakulov [1], F. N. Kurchenko et al. [5], M. Hosomani et al. [6].

Table 2
Antigenic activity of associated virus vaccine against sheep and goat pox in goats

Animal Group No.	Virus vaccine	SP и GP virus ratio in the field immunization dose (lg TCD_{50}/cm^3)	Against virus	Post vaccination antibody titer (log_2), days	
				14	21
1	SP and GP	4.24 + 4.24 (1:1)	SP	3.50 ± 0.14	3.67 ± 0.08
			GP	3.50 ± 0.29	4.00 ± 0.29
2	SP and GP	4.18 + 4.37 (1:3)	SP	3.33 ± 0.22	3.58 ± 0.08
			GP	3.72 ± 0.15	3.75 ± 0.14
3	SP and GP	4.37 + 4.18 (3:1)	SP	3.42 ± 0.22	3.67 ± 0.08
			GP	3.67 ± 0.36	3.92 ± 0.22
4	SP	4.5	SP	3.67 ± 0.08	4.00 ± 0.25
			GP	3.42 ± 0.22	3.42 ± 0.22
5	GP	4.5	SP	3.17 ± 0.08	3.58 ± 0.08
			GP	4.17 ± 0.15	4.42 ± 0.22

Table 3
Virus-neutralizing activity of sera from farmed sheep and goat following immunization with associated vaccine

Animal Group No.	Animal species	Antibody titer to pox virus (log_2) after vaccination, days			
		sheep		goat	
		21	90	21	90
1	Sheep	2.853 ± 0.077	3.017 ± 0.102	3.662 ± 0.057	3.150 ± 0.111
2	Goats	3.050 ± 0.122	3.068 ± 0.107	3.417 ± 0.072	3.386 ± 0.098

The immunological value of the associated vaccine is assessed based on the level of antibodies against antigens included in the preparation, in comparison with the specific activity of blood sera from animals vaccinated with monovalent vaccines, as well as infected with virulent strains of sheep pox virus.

The optimum ratio of antigens in the associated vaccine guarantees its high effectiveness in preventive immunization of animals against infectious diseases.

The aim of the studies was to determine the antigenic and protective activity of the associated virus vaccine based on attenuated strains of sheep pox and goat pox viruses.

MATERIALS AND METHODS

Attenuated sheep pox (SP) ARRIAH virus strain and goat pox (GP) ARRIAH 2003 virus strain grown in a continuous monolayer cell culture of capra hircus gonad YDK-04 were used for formulation of the experimental associated virus vaccine. The cultivated virus was tested for sterility and infectivity in YDK-04 cell culture by tenfold serial dilutions, which was expressed in lg TCD_{50}/cm^3. The above-mentioned cultivated virus strains with the same titer of 7.0 lg TCD_{50}/cm^3 were used in the experiment. The SP and GP virus strains were mixed at 1:1 with the virus titer of 4.24 and 4.24 lg TCD_{50}/cm^3 in the preparation, at 1:3 with the virus titer of 4.18 and 4.37 in the immunization dose lg $TCD_{50}/cm^3$and 3:1 with the titer of 4.37 and 4.18 lg TCD_{50}/cm^3 in the immunization dose, respectively. The specific activity of the associated vaccines was compared with that of monovalent vaccines against SP and GP with the virus strain titer in the preparation at 4.5 lg TCD_{50}/cm^3. A stabilizer was added to each of these preparations, then filled in 4.0 ml vials, lyophilized, the vials were filled with dry sterile air, covered with rubber stoppers and rolled with metal caps. The prepared experimental associated and monovalent virus vaccines were stored at 4.0 ± 0.5 °C until the studies were carried out.

The study of antigenic and protective properties of the virus vaccine was carried out using 32 animals of 2–3 years of age: 17 sheep of the Romanov breed and 15 goats of the local breed, selected according to the principle of analogues and not demonstrating antibodies against sheep and goat pox in the blood. Each vaccine was administered to 6 animals per group (3 heep and 3 goats in each group). The vaccines were diluted with PBS (pH 7.4) taking into account 100 immunization doses in a vial and then injected subcutaneously into the inner thigh area at 1.0 cm^3.

Antigenicity of the virus vaccine was determined based on results of blood sera tests obtained on day14 and 21 post vaccination by microneutralization test in YDK-04 cell culture against 100 TCD_{50}/cm^3 homologous and heterologous viruses of SP and GP. The antibody titer was expressed in log_2.

On day 21 post vaccination all sheep were inoculated with a suspension of the SP virulent virus Afghan strain by injecting 500 ID_{50} subcutaneously into 0.5 cm^3 in the area of the tail fold. Two non-immune sheep were used as controls. The results of challenge tests were assessed for 13 days (observation period) taking into account the presence of specific pox lesions on the skin.

RESULTS AND DISCUSSIONS

The administration of an associated virus vaccine did not adversely affect the physiological state of the animals and was accompanied by the formation of virus neutralizing antibodies in protective titers that did not differ from antibody titers in the blood of sheep and goats during monovalent immunization. No significant differences were observed in the specific activity of blood sera of sheep and goats immunized with associated viral vaccines containing antigens of SP and GP virus in the immunization dose, which is apparently related to the presence of high titers of attenuated sheep pox virus of at least 4.0 lg TCD_{50}/cm^3 in the field immunization dose.

Thus, the antibody titer in sheep blood on days 14 and 21 after inoculation with the associated virus vaccine at 1:1 ratio of SP and GP virus antigens in the field immunization dose, was 3.00 ± 0.21 and 3.63 ± 0.13 log_2 against SP virus, and 3.27 ± 0.21 and 3.50 ± 0.50 log_2 against the GP virus (Table 1).

The specific activity of blood sera from sheep vaccinated with an associated virus vaccine containing SP and GP virus antigens at 1:3 ratio in the immunization dose was characterized by the presence of a protective titer of antibodies to the SP virus in the same periods 3.17 ± 0.31 and 4.00 ± 0.50 log_2, and to the GP virus – 3.33 ± 0.31 and 3.51 ± 0.98 log_2. Similar results were obtained when the animals were administered with a virus vaccine containing SP and GP virus antigens at 3:1 ratio. Antibody titers in the blood in the indicated periods were within the range of 3.33 ± 0.12 and 4.05 ± 0.22 log_2 and 3.25 ± 0.20 and 3.87 ± 0.12 log_2, respectively.

Differences in the activity of blood sera of sheep, immunized with homologous and heterologous monovalent SP and GP virus vaccines were detected. Thus, on day 14 after immunization of sheep with the virus vaccine containing 4.5 lg TCD_{50}/cm^3 of SP homologous antigen in the immunization dose, the antibody titer was 3.62 ± 0.12 log_2, and to the heterologous GP virus – 2.50 ± 0.50 log_2.

Immunization of sheep with GP virus vaccine induced formation of antibodies, the level of which was slightly

higher against GP homologous virus – 3.12 ± 0.37 and 4.17 ± 0.23 log_2, and against SP heterologous virus – 2.75 ± 0.25 and 3.75 ± 0.25 log_2. Minor differences in the specific activity of blood sera may occur due to the broader antigenic spectrum of GP virus compared to SP virus.

It was found that at day 21 all sheep immunized with associated vaccines and a monovalent vaccine against SP were resistant to sheep pox when infected with the SP virulent virus AfGHAN strain, whereas all the three sheep vaccinated with a GP monovalent vaccine demonstrated the presence of an extensive local reaction in the form of edema and hyperemia of 3×5 cm at the site of virus suspension inoculation, and one sheep showed a generalized pox form. Two control sheep were diseased with pox in a generalized form with the formation of papules and pustules on the muzzle in the area of the lips and nose, as well as in the hairless areas of the fore and hind limbs.

The results of studying the antigenic activity of the associated vaccines in goats are almost identical to those in sheep (Table 2). No negative effects on antigenic activity of attenuated strains of the SP and GP virus in different ratios in the vaccine dose were identified.

Thus, with the same ratio (1:1) of SP and GP attenuated viruses in the field immunization dose, antibody titers in goats to SP and GP viruses on day 14 post vaccine administration were 3.50 ± 0.14 and 3.50 ± 0.29 log_2, and on day 21 – 3.67 ± 0.08 and 4.00 ± 0.29 log_2, respectively (Table 2).

The 1:3 ratio of vaccine strains in immunization dose after vaccination of goats was accompanied by the formation of virus neutralizing antibodies to SP and GP pathogens in the indicated periods in titers 3.33 ± 0.22 and 3.72 ± 0.15 log_2, as well as 3.58 ± 0, 08 and 3.75 ± 0.14 log_2.

Similar results were obtained after inoculation of an associated vaccine containing SP and GP viruses at 3:1 ratio in the field immunization dose. Within the same period the antibody titers were 3.42 ± 0.22 and 3.67 ± 0.36 log_2, as well as 3.67 ± 0.08 and 3.92 ± 0.22 log_2.

Immunization of goats with monovalent vaccines containing 4.5 lg TCD_{50}/cm^3 of SP and GP attenuated strains in the field immunization doses was accompanied on day14 and 21 by the formation of antibodies in blood against homologous and heterologous viruses in high titers: 3.67 ± 0.08 – 3,17 ± 0.08 and 4.00 ± 0.25 – 3.58 ± 0.08, 3.42 ± 0.22 – 4.17 ± 0.15, and 3.42 ± 0.22 and 4, 42 ± 0.22 log_2, respectively.

Further studies were conducted with regard to efficacy of the associated SP and GP virus vaccine of the production batch under practical conditions. For this purpose the vaccine was administered to sheep and goats in private households according to the manufacturer's instructions and the vaccine antigenicity was studied by microneutralization test based on the titers of virus neutralizing antibodies in the blood following immunization of animals. A total of 120 samples of sheep and goat sera obtained by 30 samples on days 21, 90 and after vaccination of animals were tested (Table 3). The formation of antibodies to SP and GP viruses after immunization of animals of both species was established, the level of which in the blood of sheep was high and corresponded to 2.853 ± 0.077 and 3.017 ± 0.102 log_2 to the SP virus, 3.662 ± 0.057 and 3.150 ± 0.111 log_2 to GP virus, and in the blood of goats – 3.050 ± 0.122 and 3.068 ± 0.107 log_2 to the SP virus, and to the GP causative agent – 3.417 ± 0.072 and 3.386 ± 0.098 log_2 at days 21 and 90 respectively.

CONCLUSION

Experimental associated virus vaccines containing 4.24 + 4.24, 4.18 + 4.37 and 4.37 + 4.18 lg TCD_{50}/cm^3 attenuated strains of SP and GP viruses demonstrated pronounced antigenic and protective properties. No negative effects on the formation of immunity have been noted after vaccine administration in sheep and goats. It may be associated with the presence of at least 4.0 lg TCD_{50}/cm^3 of each antigen in the field immunization dose, which significantly exceeds the field immunization dose recommended by the "Guidelines for Diagnostic Tests and Vaccines for Terrestrial Animals (OIE)" [9] equivalent to 2.5 lg TCD_{50}/cm^3.

The associated virus vaccine against sheep and goat pox in the production batch appeared to have pronounced antigenic properties when used under practical conditions.

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