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AEROSOL VACCINATION OF GUINEA PIGS AGAINST ANTHRAX

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Modern prevention of infectious diseases is involving more and more mass vaccination of animals by which the susceptibility of the animals to the infection is reduced. Among many methods of vaccinations of animals (skin vaccination, subcutaneous, intramuscular, oral, etc.), the aerosol method is gaining in interest. It makes it possible to immunize simultaneously a large number of animals within a very short period of time (in about 15 minutes) by using a small amount of vaccine. The preparation is introduced in the respiratory organ in the form of aerosol. It affects a very large surface of the tissue of the organism and a large number of the nervous reception organs, because the respiratory area of the animals is 60-65 times larger than the surface of the skin and several hundred times larger than the surface of the stomach. At the same time, in the case of aerosol vaccination we have spontaneous combination of immunization through the lungs, through the conjunctiva, and through the mouth.

Aleksandrov and associates (1, 2) carried out interesting studies concerning immunization of animals by means of sprays of dry vaccines against anthrax, brucellosis, tularemia, and plague. Their experiments covered 500 guinea pigs, 165 rabbits, 523 sheep and 30 monkeys, which were immunized in small chambers 1.5-5 m² in capacity, or in rooms 5-20 m³ in capacity. For control purposes, they vaccinated animals subcutaneously in the laboratory. The resulting degree of immunization as well as the period of immunization after the application of the method of aerosol vaccination were similar to the results observed in subcutaneous vaccination.

In the study by Eigelsbach and associates (4) relating to the vaccination of monkeys and guinea pigs against tularemia, the description gives the results of the observations of the degree of immunization after

vaccination by several methods. Monkeys vaccinated by the aerosol method showed greater immunization to the infection by microbes of tularemia through aerosol than monkeys immunized intradermally. In the first case 100% of the monkeys which were infected by a dose of 750 live bacilli survived, in the second case 57-63% of them survived.

At the same time, in the case of the animals of the first group it was possible to breed infectious bacilli of tularemia from the blood in 13% of the cases, in the second group in 50-71%. The authors conclude that the aerosol immunization by means of vaccine against tularemia results in greater resistance than intradermal or subcutaneous vaccination. White and associates (13) studied the dynamics of the formation of antibodies in monkeys which have been vaccinated against tularemia by the aerosol method and by the intradermal method. Among the animals of the first group they found antibodies in the cells of the lung tissue as early as seven days after the vaccination, while in the second group they did not find them at all in such cells, and they were able to discover them two weeks later at the place of vaccination. The dynamics of the formation of antibodies in the regional lacteal glands and in the spleen of both groups of animals was the same.

Fritulin (9) made studies concerning the possibility of aerosol vaccination of 25 sheep, 12 calves, 3 pigs, 3 horses, and 3 goats against paratyphoid fever. He carried out the experiments in rooms in which he sprayed vaccines in the amount of 30-40 million microbes in one liter of the air. The average dose of the aerosol per animal was 500-600 million bacteria during an exposure lasting 15-30 minutes. The animals under study achieved immunity after 8-10 days, and the immunity lasted 10-11 months.

Mieszczerekowa (8) tried to immunize 8 pigs by means of vaccine against dysentery. The vaccine was sprayed in the place where the animals were located. She achieved a high degree of immunity to infection by dysentery among the vaccinated animals during her experiments. Kuliesko and associates (6, 7) carried out studies of swine plague. They immunized 19 pigs by aerosol vaccine. In 9 of them they obtained strong immunity against the plague, while in the others the immunity was slight. During the experiments they found that the animals had a similar post-vaccination reaction as in the case of intramuscular vaccination.

Sielivanov (12) used a large number of animals to study the possibility of using aerosol vaccination against brucellosis. He vaccinated 6576 sheep by spraying vaccination 19 in a dose of 15-30 billion bacterial cells per animal. After 15-30 days, he noted the formation of antibodies which were registered by means of positive serological reactions (agglutination and OWD). The degree of immunization as well as the period of its duration was close to the results obtained after subcutaneous vaccination of animals.

Borsenkow (3), Prokofiewa and Golubniczii (10) as well as Wozniak and associates (14) carried out studies concerning the aerosol vaccination

of poultry against plague. These studies covered more than 100,000 chickens. All the experiments showed that the method of vaccination is suitable for practical application in the field.

The study described in this article deals with aerosol vaccination of guinea pigs against anthrax.

Method of Study

The experiment was carried out by means of a liquid anti-anthrax vaccine "Antraxcul" I and II manufactured by the Establishment of Bioveterinarian Industry at Gorzow. The vaccine was sprayed by means of a glass atomizer (the size of the particles was $0.88-15.72 \mu$) in a metal chamber IWK₁ with a capacity of 359 l. The guinea pigs were immunized by aerosol vaccination against anthrax I by keeping them in the chamber during the spraying which lasted 66 minutes and 36 seconds. The aerosol dose per guinea pig was equivalent to 0.1 g of the vaccine. After the exposure, the animals were transferred from the chamber to cages and the degree of their immunity was determined by means of subcutaneous administration of vaccine II, in which LD₅₀ was determined for guinea pigs by the Reed and Muench method (11). While the aerosol vaccination was in progress, another group of animals were vaccinated intradermally by a dose of 0.1 ml of the vaccine. The immunization of these guinea pigs was determined in a similar way as in the case of the previous group of animals. At the same time, the authors administered the vaccine II to guinea pigs which had not been immunized. This was done for purposes of control.

The animals which were used in the experiments came from the O. B. Sl. Wet. cattle, they were healthy, well kept, and their weight was 380-400 g.

Results of Studies

The first group of animals, which consisted of three guinea pigs, were immunized by aerosol vaccination against anthrax, three guinea pigs were immunized intradermally and three guinea pigs were used as a control group by giving them five doses of LD₅₀ vaccines II 30 days after the immunization. The same procedure was applied to the animals of the second and third group. Further studies of the degree of immunity of the vaccinated animals were carried out with the next three groups after 60 days. The amount of the vaccine was reduced to 2 LD₅₀. The last test was carried out after 90 days with a dose of 1 LD₅₀. The guinea pigs which did not show immunity died of anthrax within 2-4 days. The results obtained are presented in table 1.

Among nine guinea pigs which were immunized by aerosol vaccine, only two were immune to infection by anthrax after 30 days from the moment of vaccination, while in the group of animals which were vaccinated intradermally there were four guinea pigs which survived the infection. After 60 days, there remained only one guinea pig which was immunized among nine guinea pigs which received aerosol vaccination, while two guinea pigs were immunized in the group of guinea pigs which had been vaccinated intradermally.

Table 1. Degree of immunity of guinea pigs vaccinated against anthrax by the aerosol method and intradermal method.

Tab. 1. Stopień odporności świnek morskich uodpornionych przeciw wąglikowi metodą aerozolu i intryderskorną

(1)	(2) Po 30 dniach		(3) Po 60 dniach		(4) Po 90 dniach		
	3 LD ₅₀		2 LD ₅₀		1 LD ₅₀		
	uodpornian (5)		uodporniane (5)		uodporniane (5)		
Liczba zwierząt	6) aerosol	7) intrydersk.	6) aerosol	7) intrydersk.	6) aerosol	7) intrydersk.	8) kontrol.
	75	31 ² 32	33	3/3 33	33	3/2 11	33
	24	33 31	3/3 3/2	3/2 33	33	1/1 2/1	33
24	33 32	3/3 33	32 33	33	1/1 22	33	

¹⁾ Liczba oznacza liczbę zwierząt użytych w doświadczeniu, mianownik liczbę zwierząt padłych.

Key: 1 - Number of animals, 2 - after 30 days, 3 - after 60 days, 4 - after 90 days, 5 - immunized, 6 - aerosol method, 7 - intradermal method, 8 - control.

Note*: The numerator designates the number of animals used in the experiment, the denominator indicates the number of animals which died.

Studies of five animals immunized by aerosol vaccination and five by intradermal vaccination after 90 days from the moment of vaccination showed immunity to anthrax only in the case of one guinea pig both in the first group as well as in the second group. All animals vaccinated by anti-anthrax vaccine II for purposes of control died of anthrax.

Description of Results

It is understandable that the modern, fast, and economic method of aerosol vaccination of animals against infectious diseases arouses general interest. It requires instruments which are not very complicated and can be applied in normal closed accommodations for a large number of animals. However, our own studies concerning the possibility of using aerosol vaccine against anthrax on guinea pigs showed that the method is less effective than intradermal vaccination. This is shown in table 2.

Table 2. Comparison of immunity to anthrax of guinea pigs which have been given aerosol and intradermal vaccination.

Method of vaccination	Number of vaccinated animals	Animals immunized against infection	
		Number	Percent
Aerosol	23	5	21.7
Intradermal	23	7	30.4

The table shows that intradermal vaccination protected about 1.5 times as many animals against infection by anthrax as the aerosol method. True,

the results obtained are less advantageous than the results given by Aleksandrova and associates (1, 2). Nevertheless, they indicate that antibodies are formed in the organism of guinea pigs which received aerosol vaccination. At the same time, the smaller proportion of immunization by aerosol vaccination of small laboratory animals in comparison to the intradermal method could be due to the possibility that the aerosol was not homogeneous. The particles of the aerosol reached the size of 0.88-15.72 . On the other hand, particles larger than 10^4 do not reach the pulmonary vesicles. Instead, they remain in the upper respiratory passages and a great deal of them are eliminated from the organism (5).

Simultaneous immunization of guinea pigs by aerosol vaccination and by intradermal vaccination against anthrax resulted in immunity to the infection of a small number of animals under study (21.7-30.4%). We can assume that guinea pigs show little immunological reaction when they are given vaccination against anthrax both intradermally as well as through the lungs.

The studies described above were carried out on a small number of guinea pigs and only on one species of laboratory animals. At the same time, the study represents only part of a larger problem of aerosol vaccination. Consequently it requires further experiments with various vaccinations and a broader processing involving a larger amount of animal material.

Conclusions

1. Aerosol immunization of guinea pigs against anthrax proved less effective than intradermal vaccination.
2. The method of immunization of animals against anthrax by aerosol vaccination could be used in practice after more extensive experimental tests.

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