



## Immunogenic Activity of a Polyvalent Radiovaccine Against Colibacteriosis, Salmonellosis and Pasteurellosis of Farm Animals.

<sup>1</sup> Mirzaev B.Sh.

<sup>1</sup> Applicant Uzbek Research Institute of Veterinary Medicine, Samarkand, Republic of Uzbekistan.

**Abstract:** With the help of radioactive biotechnology, a new polyvalent radiovaccine against pasteurellosis, salmonellosis and colibacillosis of farm animals has been developed. The vaccine is highly immunogenic. Its use prevents diseases and death of lambs, calves and piglets from pasteurellosis, salmonellosis and colibacillosis.

**Keywords:** Animals, radiovaccine, strain, pathogen, bacterial diseases, antigen, antibodies, blood serum, resistance, immunity.

### Introduction

It is well known that, to obtain vaccines used in veterinary medicine, the methods of physical (heating, cooling, etc.) and chemical (formalin, etc.) effects on vaccine strains of animal pathogens are most often used. But with such exposure, the antigenic structure of the cells of pathogens responsible for the creation of immunity is destroyed. Applied radiobiology involves the use of the results of fundamental studies of phenomena that can serve as the basis for the development of certain technological processes. The use of ionizing radiation to influence bacteria and viruses makes it possible to maintain high immunogenic properties while reducing their virulent properties. (D.A. Kaushansky; A.M. Kuzin 1984).

Vaccination of farm animals against several infectious diseases at the same time is of not only theoretical, but, what is very important, of great practical interest, since the terms of vaccination are significantly reduced and the material costs of immunization are significantly reduced.

Taking into account the epizootic situation of pasteurellosis among sheep and livestock in Uzbekistan over the past 25-30 years, often in the form of both monoinfection and in a mixed form of manifestation, a radiation biotechnology for the design of radiovaccines against bacterial agricultural diseases was developed in the laboratory of radiobiology of the Scientific Research Institute of Veterinary Medicine. (Bulkhanov R.U., Ryasnyansky I.V., Mirzaev B.Sh. 1999, 2001, 2004). Radiation biotechnology makes it possible to create and produce both mono- and polyvalent vaccines for veterinary medicine. Using this biotechnology, we have developed and put into practice "Associated radiovaccine against colibacillosis and salmonellosis of calves", "Associated radiovaccine against colibacillosis and salmonellosis of small ruminants" and "Polyvalent radiovaccine against colibacillosis, salmonellosis and pasteurellosis of farm animals".

The purpose and tasks. The purpose of these studies was the development and production testing of a polyvalent radiovaccine against three infectious diseases in young farm animals. The objectives of the research were to study the properties and effectiveness of the polyvalent vaccine in laboratory conditions in experiments on lambs, calves and piglets, as well as to study the immunogenic effectiveness of this vaccine in the production conditions of farms.

Materials and research methods. Experimental batches of a polyvalent vaccine containing 17 vaccine strains (E.colli-8, Salmonellae-4, Pasterellae-5) of the causative agents of these diseases in young

animals were made according to the radiation biotechnology developed at the Research Institute of Veterinary Medicine. Biocontrol of each series was carried out.

I. Experiments on lambs. Under laboratory conditions, experiments were carried out on three groups of lambs, 8 heads in each group: lambs of the 1st group were vaccinated with PRV 1 time at a dose of 1 ml / head, lambs of the 2nd group - 2 times 1 ml with an interval of 15 days, and 3 - i group served as control.

The dynamics of immunogenesis and the effect of PRV on resistance were studied by taking blood samples after 7, 15, 30 days and then once a month for a year. The titers of antibodies in the blood serum were determined by the test-tube method of agglutination reaction with 3 antigens separately.

By studying the processes of immunogenesis, the number of T- and B-lymphocytes in the blood was controlled (M. Jondal e.a., 1972, N.F. Mendes e.a., 1973).

Resistance after vaccination was studied by determining the bactericidal (O.V. Smirnova, M.A. Kuzmina, 1972) and lysozyme (N.V. Chumachenko, L.A. Snegova, 1970) activity of blood serum.

The effectiveness of PRV vaccination in laboratory conditions was determined in an acute experiment on lambs by infecting them 6, 9, 12 months after immunization. Production tests were carried out on 21042 heads of lambs in the farms of Surkhandarya, Kashkadarya and Jizzakh regions of Uzbekistan.

II. Experiments on calves. In order to study the protective properties of the vaccine, experiments were carried out on 3 groups of calves, 8 heads each: calves of the first group were injected with 4 ml 1 time at 10 days of age; the 2nd group was vaccinated with doses of 3 and 4 ml 2 times with an interval of 15 days; the third group served as a control and was not immunized.

The effectiveness of PRV immunization was studied by conducting an acute experiment on infecting calves of the 1st and 2nd groups 1, 3, 6, 9, 12 months after vaccination. Production tests were carried out in 6 farms of the Surkhandarya region, 2 farms of Samarkand, 3 farms of Navoi and 1 farm of Jizzakh regions, in total on 1132 heads of calves.

Immunogenicity was studied by RA-determination of antibody titer in blood serum. Blood for research was taken 7, 15, 30 days after vaccination and then each time before infection (background), and after control infection after 15 days during the year. At the same time, the bactericidal and lysozyme activities of the blood were determined, and the dynamics of changes in the number of T- and B-lymphocytes was also studied.

III. Piglet experiments. Experiments in laboratory conditions were carried out on 24 heads of piglets of 3-5 days of age (3 groups of 8 heads each). Piglets of the 1st group were vaccinated with a polyvalent vaccine at a dose of 1 ml/head; animals of the 2nd group were vaccinated twice with an interval of 15 days with a dose of 1 ml, then at the age of 55-56 days, piglets of both groups were vaccinated again (before weaning) with a dose of 2 ml; Group 3 was a control group and was not vaccinated.

The effectiveness of PRV vaccination was tested by infecting piglets of the 1st group 9 months after the 1st or 7 months after the 2nd vaccination and piglets of the control group of the same age.

Production tests were carried out in 2 farms of Kashkadarya (160 heads of experimental groups and 30 heads in control) and Samarkand (35 heads in experiment and 10 heads of control) regions on 195 heads of experimental and 40 control piglets. Studies of the dynamics of immunogenesis and resistance indicators were carried out similarly to the methods described above.

Main results. Biocontrol of pilot batches of polyvalent vaccine showed that the vaccine is sterile, harmless, active and suitable for widespread use.

Experiments on the immunization of lambs have shown that already 7 days after vaccination, the titers of specific antibodies in the blood serum reach 1:600 against colibacillosis and salmonellosis, 1:500 against pasteurellosis. After 15 days, titers averaged 1:3200 against three diseases, on day 30

after a single vaccination, titers against colibacillosis were 1:1200, against salmonellosis - 1:800, against pasteurellosis 1:800. By the second month after vaccination, the titers were 1:40 against *Collies* and *Pasteurella*, 1:50 against *Salmonella*. However, with each control infection of lambs in order to determine the duration of immunity, there was a sharp surge in antibody production up to 1:2400 on average.

The results of the 2nd group subjected to double immunization showed that after the second vaccination the antibody titers averaged 1:3600, the high level of antibodies lasted a little longer than in the first group, and by the 30th day after the second vaccination reached an average of 1:1600, then by the second month after vaccination decreased to 1:50.

Immunization had a positive effect on the resistance of lambs - the bactericidal and lysozyme activity of blood serum increased, respectively, from 70% to 83% and from 24.2% to 27.8%.

After vaccination, it was also noted, at first, a slight decrease, and then an increase in the number of T- and B-lymphocytes. So on the 7th day, T-lymphocytes at a background of 2.6 thousand /  $\mu\text{l}$  decreased to 1.8 thousand /  $\mu\text{l}$ . On the 15th day after vaccination, an increase to 4.1 thousand /  $\mu\text{l}$  was noted, then the level of T-lymphocytes slowly decreased to 2.4 thousand /  $\mu\text{l}$  - 2.6 thousand /  $\mu\text{l}$  on day 45. The number of B-lymphocytes from 1.1 background increased to 2.2 thousand /  $\mu\text{l}$  on the 15th day, and on the 40-45th day it approached the norm - 1.7 - 1.3 thousand /  $\mu\text{l}$ .

Testing the effectiveness of PRV vaccination in lambs in the laboratory showed that immunity in single vaccinated lambs after 6 and 12 months is 85% and 66%, respectively, in double vaccinated 95% and 85% within a year.

Production tests at the farm named after I. Diyorov, Sariasijsky district, Surkhandarya region, for 1277 heads of lambs showed that double vaccination prevents 95% of lambs from disease and death from pasteurellosis, salmonellosis and colibacillosis. Extensive production tests in the farms of Surkhandarya, Jizzakh, Samarkand and Kashkadarya regions on a total of 21042 heads of lambs showed that during the year there was no case among vaccinated lambs, while among unvaccinated animals there was a high percentage of diseases and death from these diseases, mainly from pasteurellosis of sheep.

Experiments on calves: Immunization of calves showed that already 7 days after a single vaccination, the titers of specific antibodies in the blood serum reach 1:400 - 1:600 against colibacillosis and salmonellosis, 1:600 - against pasteurellosis. After 15 days, the titers averaged 1:2400 - 1:2800 against three diseases, on the 30th day after a single vaccination, the titers against colibacillosis were 1:400, against salmonellosis - 1:500, against pasteurellosis 1:400. By the second month after vaccination, the titers were 1:100 against *Collies* and *Pasteurella*, 1:150 against *Salmonella*. However, with each control infection of calves in order to determine the duration of immunity, there was a sharp surge in antibody production up to 1:2400 on average.

The results of the 2nd group subjected to double immunization showed that after the second vaccination the antibody titers averaged 1:3200-1:3400, the high level of antibodies lasted a little longer than in the first group and by the 30th day after the second vaccination reached an average of 1: 600, then decreased to 1:150 by the second month after vaccination.

The stimulating effect of vaccination on the resistance of calves was noted - the bactericidal and lysozyme activity of blood serum increased, respectively, from 60.2% to 75% and from 16.2% to 18.7%.

After vaccination, it was also noted, at first a slight decrease, and then an increase in the number of T- and B-lymphocytes. So on the 7th day, T-lymphocytes at a background of 1.5 thousand /  $\mu\text{l}$  decreased to 0.8 thousand /  $\mu\text{l}$ . On the 15th day after vaccination, an increase to 3.4 thousand /  $\mu\text{l}$  was noted, then the level of T-lymphocytes slowly decreased to 2.4 thousand /  $\mu\text{l}$  - 2.5 thousand /  $\mu\text{l}$  on day 45. The number of B-lymphocytes from 0.4 thousand/ $\mu\text{l}$  of the background increased to 2.6

thousand/ $\mu\text{l}$  on the 15th day, and on the 40-45th day it approached the norm - 1.1-1.0 thousand/ $\mu\text{l}$ . In those vaccinated once, in those vaccinated twice, T-lymphocytes reached 3.7 thousand /  $\mu\text{l}$ , the level of B-lymphocytes reached 3.2 thousand /  $\mu\text{l}$ .

It is known that the effectiveness of vaccination is most reliably determined by the infection of vaccinated and control animals with pathogens against which this vaccine was created. Given this, in the laboratory after 6; 9; and 12 months after a single vaccination, 3 heads of vaccinated and 2 heads of control calves were infected with a mixture of *Pasteurella*, *Salmonella* and *Escherichia*. Among the calves of the experimental groups (vaccinated), the disease and death were not noted, while the control calves fell ill and died. The effectiveness of vaccination was 100%, duration - 1 year (study period).

Conducted in 2000-2002, production tests, first on a limited livestock in 2 farms of Samarkand (168 heads) and Syrdarya (249 heads) regions, and then on a large scale - on 860 heads, showed that among vaccinated calves, the disease and death from salmonellosis, pasteurellosis and colibacillosis were not noted. At the same time, in farms where PRV was not used, the disease and death of animals were noted, as evidenced by the reports of veterinarians and requests to the institute for help in the prevention of these diseases.

Piglet vaccination experiences: carried out on 24 heads showed that in twice vaccinated piglets the number of T-lymphocytes from 0.4 thousand /  $\mu\text{l}$  (background) on days 15, 30, 60, 90 increased by 1.8; 7.2; 4.3; 1.0 thousand/ $\mu\text{l}$ , respectively. At the same time, the number of B - lymphocytes increased from 0.1 thousand /  $\mu\text{l}$  to 1.1; 1.7; 1.4; and 0.9 thousand/ $\mu\text{l}$ , respectively.

In animals of the second group vaccinated with PRV three times, changes in the number of T- and B-lymphocytes proceeded with the same dynamics, but only slightly higher than in the first group.

The study of the dynamics of immunogenesis showed an increase in antibody titers in the blood serum of vaccinated, so 15, 30, 60 days after single and double immunization, antibody titers in the blood serum of piglets were: against *Pasteurella* - 1:1600, 1:3200, 1:800, respectively, and by day 90 and beyond, the credits dropped to 1:50 and 1:25. Moreover, differences in antibody titers against different antigens in animals of the 1st group were noted only in the first days after vaccination.

Two and three times vaccinated PRV piglets showed an increase in the bactericidal activity of blood serum from 47.5% (background) to 72.3; 83.8; 78.8% at 15, 30, 45 days after vaccination, respectively. Whereas in the control these changes are obviously associated with age development and amounted to 48.4; 57.2; 65.5% respectively.

The dynamics of changes in the lysozyme activity of the blood serum of piglets is also noted, with a background of 10.7% in these periods of the study, it was 14.5; 18.3; 18.9% then dropped to 17.4; 16.1; 13.3%, while in the control with a background of 10.6% it was 11.4; 11.8; 12.9% in these terms and lower than in the experimental group. It should be noted that the difference between the indicators in two- and three-fold vaccinated was insignificant.

The study of the effectiveness of PRV was carried out on piglets of the 1st group 10 months after the 1st or 8 months after the second vaccination. At the same time, 3 heads of experimental piglets and 2 control heads were infected with a lethal dose of a mixture of *Salmonella*, *Escherichia* and *Pasteurella*. After 6 days, the control animals died with signs of a mixed infection, while in the experimental animals, disease and death were not noted.

Production tests were carried out in 2 farms of the Kashkadarya region (160 heads in the experiment and 30 in the control) and Samarkand (35 heads in the experiment, 10 heads in the control) regions for 195 experimental and 40 heads in the control. There were no cases of disease and death among the vaccinated piglets, while 8 animals (20%) from the control group died on the 5-8th day after infection.

Conclusions.

Polyvalent radiovaccine against colibacillosis, salmonellosis and pasteurellosis of agricultural animals, developed in the laboratory of radiobiology of VRI, has high immunogenic properties for one year.

Active immunogenesis in PRV-vaccinated animals is due to an increase in T- and especially B-lymphocytes in the blood and, as a result, an increase in antibody titers in the blood serum.

The positive effect of immunization on the resistance of the animal organism and active immunogenesis determined the good efficiency of vaccination and the safety of lambs, calves, and piglets.

Taking into account the positive results of the work carried out, instructions on the use of PRV for the vaccination of lambs in 1999 and for the vaccination of calves in 2001 were prepared and approved. In 2004, for this vaccine, a block of documents (TU, instructions for the manufacture and control of instructions for use) was presented and approved by the State State Budgetary Institution of the Ministry of Agriculture of the Republic of Uzbekistan.

The scope of the article does not allow to provide a large amount of material on the study of colostral immunity using PRV, where good results were obtained in passive and active immunization of pregnant animals and their offspring.

#### **List of used literature:**

1. Bulkhanov R.U., Results and prospects of radiation biotechnology in veterinary medicine // Monitoring the spread and prevention of especially dangerous animal diseases / Proceedings of scientific conference reports. dedication 10th anniversary of Independence of the Republic of Uzbekistan. and 75th anniversary of the Uzbek Scientific Research Institute of Veterinary Medicine. Samarkand. 2001 pp.36-37
2. Bulkhanov R.U. Ryasnyansky I.V., Mirzaev B.Sh. //prevention of pasteurosis, salmonellosis and colibacillosis in lambs// Problems of creation, synthesis and production of drugs for veterinary medicine/ Collection of materials of the 3rd scientific-practical conference. Samarkand. 2004 Page 25.
3. Kaushansky D.A., Kuzin A.M.//Radiation biological technology.//Energoatomizdat. M.1984, p. 152.
4. Mirzaev B.Sh., Yuldashev R.Yu., Bulkhanov R.U.//Indicators of T- and B-lymphocytes in the blood of calves vaccinated with a polyvalent radiovaccine//Monitoring of the spread and prevention of especially dangerous animal diseases//Proceedings of scientific conference reports devoted to 75 anniversary of the Uzbek Scientific Research Institute of Veterinary Medicine. Samarkand. 2001 pp 98-100.
5. Ryasnyansky I.V., Bulkhanov R.U., Mirzaev B.Sh., Avakyan B.G.// The effect of polyvalent radiovaccine on the qualitative and quantitative indicators of leukocytes in piglets and pregnant sows// Problems of creation, synthesis and production of drugs for veterinary medicine / Collection of materials of the 3rd scientific-practical conference. Samarkand. 2004. pp. 65-66.
6. Mirzaev B.Sh., Bulkhanov R.U., Ryasnyansky I.V.\\ Immunogenesis in calves depending on the scheme of vaccination against colibacillosis and salmonellosis.// Problems of creation, synthesis and production of drugs for veterinary medicine/ Collection of materials of the 3rd scientific - practical conference. Samarkand. 2004. pp. 45-46.
7. Mirzaev B.Sh. //Protective activity of the associated radiovaccine against colibacillosis and salmonellosis in calves.// Monitoring the spread and prevention of especially dangerous diseases of animals and birds.// Proceedings of the Scientific Conference. Samarkand. 2006 Page 207-209.