Part 1. Veterinary medicine

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THE DYNAMICS OF THE SPECIFIC IMMUNITY FORMATION IN SHEEP UNDER THE INACTIVATED VACCINES AGAINST CONTAGIOUS AGALACTIA OF SHEEP AND GOATS

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Summary. The aim of the research is constructing and efficiency studying of inactivated vaccine against contagious agalactia of sheep and goats.

The bacterial mass of *Mycoplasma agalactiae* S-11 production culture, obtained on artificial media, has been used as initial product. The vaccine contains 60% of formalin-inactivated mycoplasmas cell suspension (6×10^7 cfu in a dose) in sterile phosphate-buffered saline and 40% of aluminum hydroxide. Vaccination of sheep in the sheep farms of Odessa region by 'Ahavak' vaccines (S.N. 'Institutul Pasteur' S.A. Romania) and NSC 'IECVM' using different schemes. Animals were studied using bacteriological, serological and biochemical methods.

The vaccine from *Mycoplasma agalactiae* S-11 strain does not cause the local reaction and immunosuppression on organisms of sensitive livestock animals. The level of protective antibodies among research groups of animals for NSC 'IECVM' vaccine was 3.54 (3.47–3.61) $\log_2$ at the 30th day after revaccination and 3.59 (3.52–3.64) $\log_2$ for 'Ahavak' vaccine. The antibodies against *Mycoplasma agalactiae* were registered in 14 heads of the control group (n=50) and accounted for 1.31 (0.00–2.86) $\log_2$.

It was found that disease signs and presence of pathogen are absent in conjoined holding animals which were vaccinated against contagious agalactia of sheep and goats by NSC 'IECVM' and Romanian vaccines as compared to the animals with clinical sings of the disease. The intracutaneous injection of inactivated vaccine against contagious agalactia of sheep and goats (NSC 'IECVM') two times provides 100% protection of sensitive animals against clinical signs of the disease. It is harmless, areactogenic, with immunogenic and protective properties equally to the Romanian vaccine.

Keywords: contagious agalactia, sheep, goats, inactivated vaccines, ELISA

Introduction. Contagious agalactia of sheep is widespread in countries with developed sheep farming (Turkey, Spain, Italy, etc.). The pathogen (*Mycoplasma agalactiae*) can circulate in group of sensitive animals during some years. The disease runs in subclinical form, but if there is stock infection more than 70%, the outbreak of clinical signs of disease happens (the peak season falls at lambing). The most sensitive are animals under lactation and young ones. Nowadays, the contagious agalactia is registered in some districts of Odessa region in Ukraine. However, due to the fact that sheep farming is actively developing, the disease can spread in other regions also. The developing of homeland biological products for diagnosis and preventing the contagious agalactia is an actual field of research (Ariza-Miguel, Rodríguez-Lázaro and Hernández, 2012; Kumar et al., 2014; Madanat, Zendulková and Pospíšil, 2001; Poumarat et al., 2016; *Veterinary Record*, 2014).

According to the OIE, the commercial vaccines against contagious agalactia of sheep, caused by *M. agalactiae*, and inactivated with formalin, are widely used in South Europe. Some researches consider that

they are ineffective. In vitro, the vaccines against *M. agalactiae*, inactivated with saponin or phenol, have more protective effect that formalized ones. Alive vaccines against *M. agalactiae* are used in Turkey, where they are more efficient, as it is reported, than inactive ones.

Materials and methods. The bacterial mass of *Mycoplasma agalactiae* S-11 production culture, accumulated in liquid culture media during 3 serial passages (72 h at 37°C), was used as an initial product. Vaccine was obtained by the following method: bacterial mass of the micoplasmas was inactivated by adding 1% formalin (24 h at 37°C). Inactivated cells were precipitated by centrifugation and washed twice with sterile PBS (centrifugation mode 3 ths.rev./min for 20 min). The suspension with 1×10^8 cfu/sm^3 concentration was prepared. Aluminum hydroxide is added to bacterial mass. The component ratio of vaccine is following: 60% of *Mycoplasma agalactiae* production strain inactivated cell suspension (6×10^7 cfu in one dosage) in sterile phosphate-buffered saline and 40% of aluminum hydroxide.

Experiments on sheep conducted under production conditions in sheep farm of Odessa region. There was dedicated a group of 150 animals from a herd. It was reformed in three groups with 50 animals in each one:

1. Intact group (50 animals) with no vaccination.

2. Research group (50 animals) vaccinated with inactivated vaccine against infectious sheep and goal agalactia (NSC 'IECVM') intracutaneously in the tail fold twice in the dosage of 1 sm^3 per 30 days.

3. Research group (50 animals) vaccinated with inactivated vaccine against infectious sheep and goal agalactia 'Ahavak' (S.N. 'Institutul Pasteur' S.A., Romania) intracutaneously in the tail fold twice in the dosage of 1 sm^3 per 30 days.

The blood of all animals was sampled for serological and biochemical studies before the immunization and on 30th days after second vaccination. The biological material (swabs from nares and eyes, milk samples) of animals from both groups was sampled and studied with bacteriological method for *Mycoplasma* presence before experiment beginning. The γ-globulins level in blood serum samples of intact and research groups was detected using standard methods. Nasal and eyes swabs were sampled from 10 animals in each of three groups for bacteriological studying on the presence of infectious agalactia pathogen at the end of the research.

Results. The clinical signs of disease and side-effects in the place of injection (reddening, swell) were absent during the studying of *Mycoplasma agalactiae* S-11 formalin-inactivated epizootic culture protective properties on sheep. Mycoplasmas were extracted from two animals after bacteriological studying of biological material from investigated animals (nasal and eyes swabs).

We conducted biochemical analyses of sheep serum samples from intact and research groups for the purpose of studying the effect of vaccines at the general physiological animal condition. There were studied such factors as general level of proteins, seromucoids, circulating immune complexes and lysozyme. The research results are shown in Fig. 1–3.

The level of lysozyme, general amount of proteins and circulated immune complexes in serum of investigated animals increased when studying. We estimated potential immunosuppressive effect of vaccine on sheep by seromuciod status. The preparation had no immunosuppressive effect on sheep; the seromucoid level almost hadn't changed after vaccination in intact and research animals.

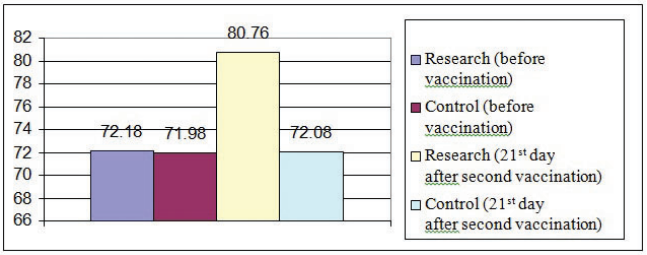


Figure 1. The dynamics of general protein level in sheep serum from control and research groups (g/l)

Injection of *Mycoplasma agalactiae* S-11 epizootic culture vaccine increased the level of general protein amount and circulating immune complexes in sheep serum of research group at 10% and 13% respectively.

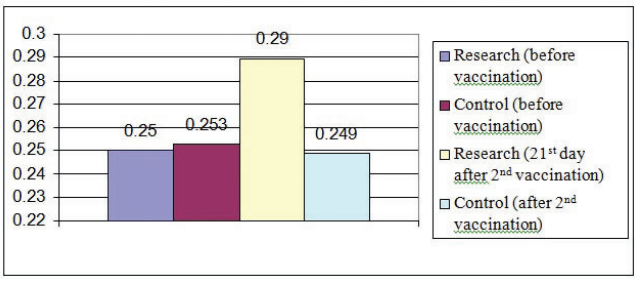


Figure 2. The changing level of circulated immune complexes in sheep serum of control and research groups (mg/ml)

The lysozyme level in sheep blood serum characterizes the level of non-specific antibacterial resistance of organism and it can have no direct connection with effects of immune medication. However, the results at the Fig. 3 show, that the lysozyme level among vaccinated animals increases. At that time the lysozyme level in control group has been lower,

that in the research one. We explain this fact that animal sensibilization with mycoplasma agent activates functioning of the specific and non-specific pathways intensity.

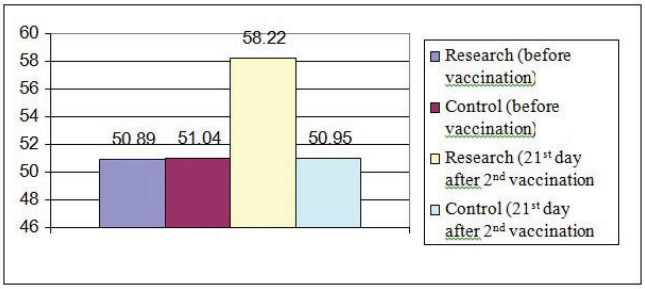


Figure 3. The dynamics of lysozyme level in sheep blood serum of research and control group (mkg/sm^3)

So, it has been determined, that experimental batch of vaccine from *Mycoplasma agalactiae* S-11 formaline inactivated culture is sterile and safe for anumals. It causes protection of 90% immunized animals after double vaccination in the challenge experiment with lethal dose of *Mycoplasma agalactiae* S-11 epizootic strain. Vaccine has no imunosupressive effect for animals and doesn't cause local allergic or inflammatory lesions in vaccinated animals.

To determine the immunogenic properties of the vaccine research was conducted on sheep in a production sheep-breeding farm in the Odessa region. Sheep were divided into groups and treated as planned experiment.

Biological material samples (nasal and conjunctive swabs, milk samples) from the experimental animals were examinated for infectious agalactia of sheep and goats agent by bacteriological method. The mycoplasmas were not detected.

After 30 days after the second vaccination of animals of all groups were selected for blood and serum biochemical studies. At the end of the experiment, 10 animals from each of the three groups were selected nasal and eye swabs for bacteriological research for the presence of infectious agalactia. In the group vaccinated animals for signs of disease were not found.

The reactogenic properties study of the vaccine against *Mycoplasma agalactiae* demonstrated their absence during all experiment period.

We occurred 12 sheep with clinical signs of suppression, watering, and swelling of the knee in the herd. Thick animals contacted with two groups of the vaccinated animals and intact sheep. At the end of experiment 5 sheep from the non-vaccinated animal group demonstrated general depression and watering. Both of them were treated by macrolide antibiotics, and the therapy demonstrated effectiveness.

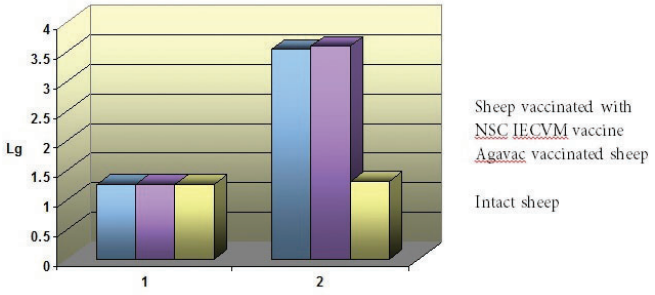
We didn't isolate agent from vaccinated animals after 5 blind passages of the clinical specimens from vaccinated animals. *Mycoplasma agalactiae* was isolated from 6 animals of the intact sheep group.

The study of immunogenic properties of *Mycoplasma agalactiae*, which is the main antigenic component of NSC 'IECVM' vaccine were tested in the experiment in sheep (Fig. 4).

The antibody titer among vaccinated animals was 3.54 (3.47–3.61) $\log_2$ for NSC 'IECVM' vaccine and 3.59 (3.52–3.64) $\log_2$ for vaccine 'Agavac' in 30 days after immunization. 14 intact animals of the control group (n=50) demonstrated antibody level 1.31 (0.00–2.86) $\log_2$.

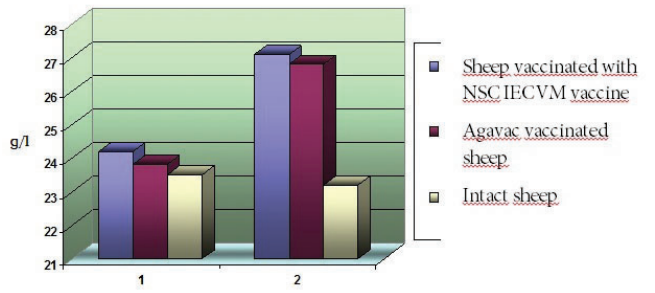
The vaccinated and intact animals' sera were also tested for biochemical parameters to study the non-specific immune response dynamics. We tested the level of γ-globulines before immunization and in 30 days after revaccination (Fig. 5).

It was demonstrated, that inoculation of both NSC 'IECVM' and 'Agavac' vaccined stimulated growing of γ-globulines levels for 10.7% and 11.1% respectively. At the intact animals group their level was more or less constant — 23.2 g/l.



1 – blood sera testing before experiment; 2 – blood sera testing at 30th day after immunization.

Figure 4. Antibody titers for infectious agalactia agent in blood sera of experimental animals of treated and control groups (Me, %25–%75, n=10)



1 – blood sera testing before experiment; 2 – blood sera testing at 30th day after immunization.

Figure 5. γ-globulines level in blood sera of vaccinated and non-vaccinated animals (Me, %25–%75, n=10)

The inactivated NSC 'IECVM' vaccine against infectious agalactia of sheep and goats is unreactogenic in conditions of the double subcutaneus administration. This preparation develop 100% protection in vaccinated animals from clinical disease.

Vaccinated animals by 'Agavac' (Romania) and NSC 'IECVM' vaccine, kept with non-vacicnated animals with clinical signs are protected from the infection.

Conclusions. The simultaneus keeping of animals vaccinated by 'Agavac' (Romania) and NSC 'IECVM' vaccine, and non-vacicnated animals with clinical signs demonstrates protection of immunized sheep from clinical infection with infectious agalactia. The double subcutaneus administration of the inactivated NSC 'IECVM' vaccine provide 100% protection of the susceptible animals. The preparation in safe, sterile, areactogenic and have immunogenic properties equal with Romanian vaccine 'Agavac'.

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