

Research Article



Biotechnology of the New AI-Test - an Indicator for the Diagnosis of Subclinical Mastitis and an Indication of the Effectiveness of the Bactericidal Drug Dioxinor for Serous Mastitis in Lactating Sheep

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Abstract | The article is devoted to the study of the effectiveness of the AI-test indicator for rapid diagnosis of mastitis in ewes. In laboratory studies using the AI-test diagnosticum, the main criterion for the presence of subclinical mastitis in animals was the formation of a well-formed purple jelly-like clot (moderate to dense) of in the milk samples, which was partially (+) or entirely (++) of thrown of out the from the hole in plate with stirring. On the plate, with a negative reaction (-), the mixture of milk and the indicator remained homogeneous, pale raspberry colored, with a dubious (+) - it contained traces of the formation of the color of raspberry jelly. To detect latent mastitis in sheep, we proposed the rapid test of the A1 test, which is an aqueous solution of sulfanol and cresol red. Unlike a 2% mastidine solution, it is more sensitive. We also recommend for the treatment of serous mastitis in lactating sheep, intramuscularly administer Dioxinor at a dose of 0.1 ml / kg body weight, in twice a day until complete recovery and oxytocin, at a dose of 5 units in once a day on of the first 2 days of treatment, and also of carry out arrogant novocaine blockade according to D.D. Logvinov, twice 0.25% novocaine of solution, at a dose of 0.5 ml / kg body weight. In the experimental group, where the complex antimicrobial preparation Dioxinor was used, the effectiveness of treating serous mastitis in sheep was 96,3%.

Keywords | Ewes, Mammary gland, Subclinical mastitis, Diagnosis, Microflora, Antimicrobial preparation Dioxinor, Effectiveness.

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INTRODUCTION

One of the factors that reduce productivity and worsen the quality of milk produced by sheep is mastitis. Depending on the resistance of the animal and mammary gland, as well as the virulence of microorganisms (staphylococci, streptococci, escherichia) caused by inflammation, there are clinical or latent forms of the disease. In the first form of mastitis, clinical signs are clearly expressed (breast enlargement, soreness, lameness) and organoleptic changes

in the secretion of the udder (milk becomes watery, contains flakes, etc.). With subclinical mastitis, it is registered 3-4 times more often (Aliev, 2017; Borisov, 2013; Vasiliev, 1993) there are no clinical signs of inflammation of the udder, and it is difficult, almost impossible to diagnose sick animals by organoleptic indicators of milk. At the same time, the biochemical composition of milk changes (Urazmetova al., 2017; Schalm., 1977), the mass fraction of fat, protein, density and coagulation decreases, the content of somatic cells, chlorides, alkalis, bacterial contamination,

pathogenic and putrefactive microflora increase (Parikov et al., 2006; Shamsieva, 2017; Gebrewahid et al., 2012). Milk from animals with latent mastitis enters skim milk, which affects the quality of the latter and products from it (Bozhkov, 1987). Previous studies have shown that on the farms of the Republic of Dagestan, inflammation of the mammary gland is noted in up to 20% of the uterine population at different stages of lactation, and in 46% of cases the disease goes into clinical form, which gradually leads to atrophy of the affected udder. As a result, sheep's milk production decreases and they are rejected as economically unsuitable. For quick diagnosis of latent mastitis, we offer the AI-test indicator. The working solution is prepared in the following ratio of components: cresol red - 0.02 g; technical sulfanol - 4.0 g; water - up to 100 ml. In appearance, the AI-test is a dark red liquid, a small precipitate is allowed, it is stored in a dark place at a temperature of 5 to 25 ° C, the shelf life is one year. According to most authors, the incidence of sheep with mastitis is recorded throughout the year. The highest percentage of clinical manifestations of mastitis was noted in January - March - 45.79%, in May - July - 40.18%. Mastitis in sheep manifests itself in three forms: serous - 43.8% of cases; catarrhal - 31.1%; subclinical - 25.1%. The main causative agents of mastitis in sheep are *Staphylococcus aureus* - 21.3%, *Streptococcus agalactia* - 14.8%, *E. coli* - 4.9%, as well as their associations: of the two - 24.3%; out of three - in 15.1%. In 19.6% of cases, microflora was not isolated. In Russia, many authors at with serous mastitis of sheep recommend the use of antibacterial drugs Mastisan, Masticide, Masticur, Anolyte Neutral (ANC) and others in doses and multiplicities regulated by instructions for use for three to four days, the effectiveness of which varies between 70-86% with recovery of animals after 6-8 days from the start of therapy (Aliev, 2017; Borisov, 2013; Vasiliev, 1993; Parikov et al., 2006; Shamsieva 2017; Gebrewahid et al. 2012; Schalm et al., 1977). The aim of this work is to study the diagnostic effectiveness of the AI-test indicator to detect subclinical mastitis in sheep and to check the therapeutic efficacy of the treatment regimen for sheep serous mastitis with Dioxinor preparations at a dose of 0.1 ml / kg body weight, twice a day, oxytocin at a dose of 5 units once a day in the first 2 days of treatment in combination with arrogant novocaine blockade according to the method of Logvinov (twice with 0.25% solution of novocaine at a dose of 0.5 ml / kg body weight).

MATERIALS AND METHODS

The experiments were carried out on sheep farms of Gergebilsky, Khunzakhsky and Gunibsky regions of the Republic of Dagestan. When examining ewes, in each recess of the milk-control plate intended for the diagnosis of mastitis of small cattle (patent No. 2495645), 0.5 ml of milk was extracted from the corresponding share the udder and

was added of 0.5 ml diagnosticum. The reaction was taken into account by the degree of formation of a jelly-like clot (moderate to dense), which was half (+) or whole (++) ejected from the hole of the plate with stirring, the color of the mixture was purple. In case of a negative reaction (-), the liquid remained homogeneous, the color of the mixture was pale raspberry, and in case of doubtful (\pm), it contained traces of jelly formation, and the color of the mixture was raspberry. As a control, a 2% mastidine solution was used, designed to detect subclinical mastitis in cows. In addition, in the milk samples, the number of somatic cells was determined on a Somatos viscometer analyzer, physico-chemical parameters (fat, protein, density) on a Lactan instrument, acidity titrimetrically, pH on a universal EV-74 ionomer. When identifying microorganisms in positive milk samples, they were guided by the "Methodological guidelines for bacteriological research of milk and the secret of the udder of cows" (1983). When examining the ewes, special attention was paid to the density, texture and size of the individual udder shares, the condition of the nipples, lymph nodes and milk production. Work on testing the effectiveness of our method for the treatment of serous mastitis in sheep was carried out from 2017 to 2019 in OTF No4 of the Gunibsky district of the Republic of Dagestan on 48 heads of lactating of sheep of the Dagestan of rock 2-5 years of lactation, divided by of the principle of analogues into 2 groups (experience - control). The animals of the experimental group (n=27), patients with serous mastitis, were injected intramuscularly with Dioxinor at a dose of 0.1 ml / kg body weight, twice a day, until complete recovery and oxytocin, at a dose of 5 units, once a day, for the first 2 day of treatment. Additionally of carried of out arrogant novocaine blockade according of to D.D. Logvinov, by twice introducing of a 0.25% solution of novocaine, at a dose of 0.5 ml / kg body weight, with an interval of 48 hours. The control group of sheep (n=21) was treated with novocaine with bicillin-3, at a dose of 600,000 units three times, with an interval of 72 hours. The contents of the mammary gland 3-4 times issued a day were dispensed into separate dishes and disinfected by boiling. For the ewes during the experiment conducted daily clinical observations. The treatment results were evaluated on days 3-7 after the last injection of the drug, comprehensively, taking into account their clinical examination, laboratory examination of the secretion from the treated udder lobes. During the experiment, clinical observations and laboratory studies of milk were carried out in accordance with the regulation "Methodological guidelines for the bacteriological study of milk and the secret of the udder of cows" (1983). The research data were subjected to statistical processing by the computer program "Biometry".

RESULTS AND DISCUSSION

A total of 2354 ewes were examined. Of these, 289 heads (12.2%) gave a positive reaction with the AI-test and 227 (9.6%) with a 2% mastidine solution. Then, from the ewes with a negative (n = 5), doubtful (n = 5), positive (n = 25) and sharply positive reaction (n = 25) in the milk samples, the content of somatic cells was determined (Table 1).

Table 1: The number of somatic cells in the milk of healthy and sick sheep with latent mastitis

AI test reaction	The animals were examined, goal.	The number of somatic cells, thousand / ml
–	5	До 500
±	5	510 – 600
+	25	605 – 800
++	25	800 and higher

Note. (-) - negative reaction; (+) - positive reaction; (±) - doubtful reaction; (++) - sharply positive reaction

The content of somatic cells in the milk of healthy animals did not exceed 500 thousand / ml, with a doubtful reaction with the AI-test - 600 thousand / ml, positive - 800 thousand / ml, sharply positive - over 800 thousand / ml. The data obtained are consistent with those of V.V. Fedorov (2000). Therefore, in ewe with hidden mastitis, the number of somatic cells in the secretion of the affected lobe of the mammary gland should be higher than 600 thousand / ml. It is known that milk is a complex physico-chemical and biological fluid that maintains a stable balance of elements in their normal ratio and is the main source of nutrition of for young mammals in the first 1.5 - 2 months of life. In inflammatory processes, even in the initial stage, changes in the physicochemical composition of milk occur in the mammary gland, on which laboratory diagnostics of the secretion of the mammary gland in lactating animals is based (Table 2).

Table 2: Physico-chemical characteristics of milk of healthy sheep and with hidden mastitis

Indicators	Ewes	
	Clinically healthy (n = 5)	Patients with mastitis (n = 12)
Mass share of fat,%	6,4±0,49	4,6±0,16
Mass share of protein,%	5,7±0,10	4,6±0,11
Density, g / cm ³	1,032±0,02	1,07±0,2
Acidity, T°	22,4±1,7	17,5±0,14
pH	6,50±0,6	7,12±0,6

So, in the milk of ewes with hidden mastitis, the fat content decreased by 33.8%, protein - by 17.5%, acidity - by 4.9 °T, pH shifted to the alkaline side to 7.12, while the

density increased by 0.025 units. With such changes, the nutritional value and technological properties of milk, necessary for the production of dairy products and cheeses, naturally decrease. Feeding such milk leads to a lag in the growth and development of lambs, as well as their greater susceptibility to various gastrointestinal and respiratory diseases (Aliev, 2017). According to a number of authors (Borisov, 2013; Vasiliev VG, 1993), ewes having mastitis can serve as sources of pathogens of other infectious diseases, and sick individuals remain bacteriocarriers for a long time. With bacteriological research milk, of pathogenic and conditionally pathogenic microorganisms were found of in 93.7% of the udder shares affected by latent mastitis. Of the selected cultures, 86.7% were represented by gram-positive cocci and 13.3% - representatives of the family *Escherichia*.

The results of studying the developed treatment regimen for serous mastitis in sheep using the complex antibacterial drug Dioxinor in combination with oxytocin and of novocaine blockade, in comparison with the currently used treatment regimen in sheep farms of the republic, are given in the Table 3.

Table 3: The effectiveness of various treatment regimens for serous mastitis in sheep

Group	The number ewes of subjected of treatment, head	Recovery time, of days	Recovered has of sheep:	
			head	%
Experienced	27	3,7±0,3	26	96,3
Control	21	4,3±0,6	18	85,7

The data in the Table 3 show that therapeutic efficacy in the experimental group where the complex antimicrobial preparation Dioxinor was used was 96,3%, and in the control – 85,7%. The effectiveness of the regimen developed scheme in the experimental group was 10,6% higher compared with the control, and the recovery time in the first experimental group, compared with the control, was significantly shorter. Our studies on the diagnostic effectiveness of the AI-test indicator for quick diagnosis of mastitis in sheep, changes in the biochemical composition of milk, a decrease in the mass fraction of fat, protein, density against the background of the growth of pathogenic microflora of at subclinical of mastitis, but with other quantitative indicators, are consistent with the authors' results. New data on the effectiveness of the new treatment regimen for serous mastitis of at lactating sheep with the use of Dioxinor preparation are consistent with the methodological approaches of other authors, but differ in terms of the effectiveness of the drug.

CONCLUSION

To detect latent mastitis in sheep, we proposed the rapid test of the A1 test, which is an aqueous solution of sulfanol and cresol red. Unlike a 2% mastidine solution, it is more sensitive. We also recommend for the treatment of serous mastitis in lactating sheep, intramuscularly administer Dioxinor at a dose of 0.1 ml / kg body weight, twice a day until complete recovery and oxytocin, at a dose of 5 units, once a day on the first 2 days of treatment, and also of carry out arrogant novocaine blockade according to of D.D. Logvinov, twice 0.25% solution novocaine, at a dose of 0.5 ml / kg body weight. In the experimental group, where the complex antimicrobial preparation Dioxinor was used, the effectiveness of treating serous mastitis in sheep was 96.3%.

CONFLICT OF INTEREST

The authors declared no conflict of interest.

NOVELTY STATEMENT

The authors declare that the results obtained on the topic of the article were obtained empirically, and the reflected information is new for science in the field of immunology.

AUTHORS' CONTRIBUTION

All authors took part in the Development of a biotechnology for the half-life of a new AI-test - an indicator for the diagnosis of subclinical mastitis and an indication of the effectiveness of the bactericidal drug Dioxinor in serous mastitis in lactating sheep, collected materials, analyzed the material, participated in writing the manuscript.

Collectively reviewed the manuscript. All authors read and approved the final version of the manuscript.

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