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Professional paper

IMMUNOPROPHYLAXIS MEASURES IN COMBATING THE ENZOOTIC FORM OF CONTAGIOUS AGALACTIA IN SHEEP

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Abstract

In 2016, a disease that had not appeared for several decades appeared in two localities of the Sharr Mountains. It was mastitis accompanied by a significant decrease in milk production, up to complete drying up. This had greatly worried the sheep owners of these villages, who were traditional breeders of sheep breeding for generations. Immediately, an on-site inspection of the sick sheep followed and, according to the symptoms and inspection of the udder and milk, it was a contagious agalactia disease caused by the microorganism of the mycoplasma genus, Mycoplasma agalactiae. This was then supplemented with the commercial ELISA serological test that is available for the detection of an antibody response to Mycoplasma agalactiae.
During this study, which was carried out between 2016 and 2021, we performed immunoprophylaxis in 5321 sheep with the Romanian-made vaccine **Agalaxin** 100 ml at a dose of 1 ml per head, twice a year. The vaccine is recommended for active immunization against contagious agalactia of sheep that are clinically healthy, both in unaffected herds and in those where the disease is developing. Immunity develops 21 days after the booster vaccination. The duration of immunity is 6 months after vaccination. To prevent the spread of the disease in unaffected herds, we also recommended the isolation of sick animals and the use of zoo hygienic measures, the removal of manure and rinsing the sheep's bedding with slaked lime. The results of the first year of vaccination were quite good, where the number of animals affected by contagious agalactia had decreased to 30% of the herd.

Keywords: Contagious agalactia, Mycoplasma agalactiae, sheep, Agalaxin

1. Introduction

Contagious agalactia is an infectious disease affecting sheep and goats caused by several Mycoplasma species with the main role of Mycoplasma agalactiae (Da Massa, et. al., 1992; Bergonier, D., et. al.,1997). This is among the most serious and economically important diseases affecting small dairy ruminants. The disease causes significant economic losses from reduced milk production and shorter production life of infected animals, so it is included in the list of diseases of global importance. The disease is found in most countries with extensive sheep farming in the Balkan Peninsula region. Most Mediterranean countries are considered endemic (Corrales et al., 2007). It also occurs in endemic forms in some individual farms in the Sharr Mountain region, in the northwest of North Macedonia. Clinical signs are numerous, including mastitis associated with a drastic drop in milk production, arthritis, keratoconjunctivitis, pneumonia and septicemia, manifested in various combinations (Nicholas, R., 1995). The spread of infection in affected farms is rapid and after a short period of time can affect up to 30-85% of the animals (Campos et. al., 2013). Treatment with antibiotics for 5 to 10 days reduces the main clinical signs of the disease, but does not eliminate the carrier of the bacteria (Azevedo et al., 2006). Control of mycoplasmosis with antibiotics is of little value, however it turns the clinically recovered animal into a carrier.
Preventive measures applied in the fight against contagious agalactia are not always sufficiently effective due to insufficient knowledge of the epidemiology of the disease, the specific features

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related to the reactivity of sheep and the availability of effective vaccines against different etiological agents. In Europe, vaccination has been practiced since the 1970s (Foggie et al., 1970), but its widespread application dates back to the 1990s. Inactivated monovaccines against *M. agalactiae* are mainly used, or combinations with *M. mycoides* subsp. *mycoides* LC, M. *putrefaciens, M. capricolum* subsp. *capricolum*. However, the real efficacy of this vaccine is questionable, especially under field conditions (Nicholas, 1995; Bergonier et al., 1997). This is attributed to the high degree of antigenic variability observed in field strains of M. agalactiae (Solsona et al., 1996; Tola et al., 1996; De la Fe et al., 2006).
The diagnosis of contagious agalactia is made on the basis of epidemiological findings and the presence of clinical signs. It is relatively easy when all three typical clinical features are observed in the herd, i.e., loss of milk production and mastitis, keratoconjunctivitis and joint lesions. In both sheep and goats, signs become manifest soon after birth when lactating animals develop mastitis. The most common form is mastitis accompanied by yellow-green milk secretion. Ocular involvement can be found in only about 50% of cases. Lameness, which is common and may persist for a long time, is observed more often in males than in females. However, if only one form of the disease is present, it can be very difficult to make a clinical diagnosis. If animals only show keratoconjunctivitis, they may recover completely. In cases of secondary bacterial infection, they may even die. The lethal outcome in such cases is 15-20%.
The clinical diagnosis must be confirmed by laboratory examination, i.e., isolation and identification of the infectious agent. The best material for analysis is milk, then eye swabs, vaginal or nasal swabs, joint exudates, blood and urine. For post-mortem examination, samples are collected from the mammary gland and regional lymph nodes, pulmonary lesions and joint exudates. Mycoplasmas can also be isolated from liver, kidney and spleen tissues, but samples should be collected at the stage of bacteremia. Culturing is carried out on liquid or solid media that support the growth of mycoplasma (Lambert 1987). M. agalactiae produces colonies with centers and a phenomenon called "film and spots". The use of biochemical tests for the identification of mycoplasma is time-consuming and gives difficult to interpret results (Lambert 1987). The assessment of the effectiveness of agalactia vaccine is usually based on the assessment of specific antibody titers, based on the determination of optical density. In Europe, attention has focused on the use of vaccines with inactivated microorganisms, formaldehyde used as the inactivating agent and aluminum hydroxide or mineral oil as adjuvants (Leon Vizcaino et al. 1995, Sarris et al., 1989). Agalactia vaccines inactivated with phenols or saponins provided a higher level of protection in experimental infections compared with those inactivated by formaldehyde, sodium hypochlorite or heat (Tola et al, 1999).
Most vaccines against contagious agalactia have presented many problems with regard to effectiveness, especially under field conditions (Bergonier et al., 1997). It is considered that the ineffectiveness of some vaccines may be caused by the high level of antigenic variability of M. agalactiae strains (De la Fe et al., 2006) or the diversity of mycoplasma strains producing infections in goat and sheep populations (OIE, 2006).

2. Material and methods

The field study over 6 years was conducted in several flocks with n= 5321 sheep. The sheep were of the autochthonous breed of the Sharr breed subspecies. They were bred in stables with indoor and outdoor sections. During the winter, the animals were fed with hay, alfalfa and ryegrass, while during the other seasons they were grazed on pastures.
In the spring of 2016 in the province of the slopes of the Sharr Mountain in April, the owners of the sheep flocks reported their concern that 2-3 days after the lambs were weaned, signs of drying of the udder appeared at the peak of the highest milk production. Immediately after this announcement, we went to the enzootic province and based on clinical signs and control of the

udder and yellow-green milk, we determined that it was contagious agalactia. After this finding, we suggested that sheep without clinical signs should be vaccinated. Sheep with clinical signs of mastitis, dry udder, fever were separated and treated with tetracycline antibiotic, with the trade name Limoxin LA. The number of affected heads was about 40 sheep. Only clinically healthy animals were selected for vaccination, the number of which was 760 heads.

Table 1. Years of vaccination and number of heads

Years of vaccination	Number of vaccinated sheep
2016	760
2017	1180
2018	1049
2019	468
2020	970
2021	894
	Total heads n= 5321

The first vaccination was carried out in 2016, at the beginning of April. The animals included in the study belonged to a flock of over 800 sheep. The flock included all age and sex categories (rams, pregnant ewes, lactating ewes, reformed ewes and lambs of both sexes). According to the owners' instructions, two categories of animals were excluded from the trial: lambs under the age of three months and female ewes during the last month of pregnancy. The second vaccination was carried out after 6 months. Also, only clinically healthy animals were selected. The final number of animals included in the study this year was 760 heads.

It should be noted that the progress of the disease began to stop about three weeks after the vaccination, and the owners were recommended to transfer the sheep to pastures further away from where they are kept during the winter period. After the sheep were removed from the winter stables, they had to remove the manure and clean the bedding, even disinfecting it with slaked lime. The bedding had to remain free until the autumn months when the sheep would return to the stable. In this way, this would have an impact on the fight against the contagious agalactia disease. After the first year of vaccination, the disease was reduced to 40% of the head. That is, the vaccination and zoo hygiene measures yielded results. However, the owners were told that vaccination should be carried out every year until the disease was eradicated.

Vaccination in 2017 was carried out in two flocks of sheep. One flock was vaccinated in the last week of April and had a total of 730 heads, while the other flock was vaccinated in early May and had a total of 350 heads. Thus, a total of 1180 sheep were vaccinated. Vaccination was repeated after 6 months. Vaccination included only clinically healthy animals. The same vaccine, Agalaxin, was used for immunization.

The vaccination in 2018 was carried out in the same flocks of sheep. One flock was vaccinated in the last week of April and had a total of 679 heads, while the other flock was vaccinated in the second week of May and had a total of 370 heads, so there were 1049 sheep in the vaccination. The vaccination was repeated after 6 months. The vaccination included only clinically healthy animals. For immunization, the Agalaxin vaccine was used.

The 2019 vaccination was carried out in a different location. The study aimed to apply the vaccine against contagious agalactia to the new herd. A total of 468 sheep were included in the vaccination. The vaccination was carried out in the second week of April and included only clinically healthy animals. The vaccination was repeated after 6 months. The same vaccine was used for immunization.

Vaccination in 2020 was carried out in two flocks of sheep. Both flocks were vaccinated in the first week of May. One flock of sheep had a total of 440 heads, while the other flock had a total of 530 heads, so there were 970 sheep in the vaccination. Vaccination included only clinically healthy animals. The same vaccine was used for immunization.
In 2021, vaccination was carried out in a flock of sheep. The flock was vaccinated in the third week of April and had a total of 894 heads. Vaccination was repeated after 6 months. Vaccination included only clinically healthy animals. The same vaccine was used for immunization.
For immunization, we chose the commercially available vaccine Agalaxin vaccine, a Romanian product. The composition of the vaccine, as specified on the label of each product, was as follows:
Agalaxin vaccine: Mycoplasma agalactiae, strain S/94, with a minimum pre-inactivation titer of 10^6 CFU/ml and inducing an increase in agglutinating antibodies with at least two binary dilutions or at least 8-11 ELISA units (A.F.S.S.A. Kit) per dose. The vaccine is inactivated with formalin (≤0.5 mg) and adsorbed on aluminum hydroxide gel (2.8-3.4 mg Al2O3). The vaccine is recommended for active immunization against contagious agalactia of clinically healthy sheep and goats, both in unaffected herds and in those in which the disease evolves. Immunity is established 21 days after the booster vaccination. The duration of immunity is 6 months after vaccination.

3. Results

The first results of the vaccination carried out in sheep showed that a solid immunity had been created. From this study it is clear that from the information received from the owners, no sheep vaccinated in that year showed specific signs of the disease. Those sheep that were initially affected with the clinical signs mentioned above were isolated and kept separately throughout the first year of vaccination. Thus, according to clinical controls carried out in the field, it was found that already with the first vaccination, the number of affected animals had decreased by nearly 80%.
After this, the owners were recommended to carry out the vaccination every year. Some of them agreed and the vaccination was carried out according to the scheme. In the following years, the vaccination was carried out in the spring months, about three weeks after calving and the same was repeated after 6 months.
The vaccination was carried out for 6 years in a row. After 2121, according to the owners and the epizootiological situation, contagious agalactia did not appear. Thus, our finding that the implementation of adequate hygiene measures, cleaning and disinfection of livestock facilities, not mixing sheep with other unknown herds, together with regular vaccination over several years, yielded appropriate results in preventing the spread of the disease.

4. Discussion

Vaccines are designed to stimulate an immune response against a pathogen so that future contact with the pathogen will not provoke clinical contagious agalactia disease. The use of vaccines for the control of contagious agalactia in endemic areas is common practice. Literature data on the effect of commercial products in the field are scarce. There are even fewer data referring to the comparative evaluation of immune reactivity in sheep vaccinated against contagious agalactia.
The function of a vaccine is to stimulate an immune response against a pathogen in order to guarantee that any future contact with the same pathogen will not provoke the disease. In order for the vaccine to be complete, it must preserve the antigenic properties of the pathogen without

harming the host. It is necessary that the inactivating substances used in inactivated vaccines do not significantly alter the antigenic properties of the proteins involved in the pathogenic process. It is common practice in the preparation of inactivated vaccines to include adjuvants in order to directly enhance the immunogenic properties of the antigens. The transition from inactivated vaccines to subunit vaccines or to synthetic vaccines will be possible only after in-depth studies on the microorganism in question.

5. Conclusion

In conclusion, the formalin-inactivated vaccine and the aluminum hydroxide gel-absorbed vaccine showed significant protective efficacy. These vaccines may be of considerable value in controlling this infection in sheep and other ruminants.

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