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POTENCY EVALUATION OF TWO COMMERCIAL VACCINES AGAINST CONTAGIOUS AGALACTIA OF SMALL RUMINANTS

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Abstract

Immunoprophylaxis is the most affordable, effective and eco-friendly tool, which recommends it as the first option to control contagious agalactia in small ruminant flocks. The purpose of this study was to evaluate the immune response toward two marketed vaccines. Both products contain Mycoplasma agalactiae inactivated with formalin, on aluminum hydroxide gel. The trial has been carried out on a flock of 700 sheep. Each vaccine was administered to 250 animals according to the manufacturer instructions and 200 animals were in the control group. Serum samples were collected on vaccination days (0 and 21) and post vaccination, at 30, 90, 180 and 360 days. The immune response was assessed using a commercial indirect ELISA kit for antibody detection. Antibody titers increased rapidly after vaccination, reached the highest level between 21 and 30 days and declined after 180 days. No statistically significant differences in titers were identified between the two vaccines.

Key words: *Contagious agalactia, ELISA, Mycoplasma agalactiae, small ruminants, vaccine.*

INTRODUCTION

Contagious agalactia of sheep and goats is a transmissible disease, first described by Metaxa in 1816, in Italy, being called "mal de sito" which means "disease of the place" because of its persistence in the environment and ability to contaminate newly introduced flocks (Jaÿ & Tardy, 2019). Initially confined to the Mediterranean basin, the disease has spread through sheep trading and population migration, nowadays being reported on every continent (Lambert, 1987; Manzat, 2001). In Romania, contagious agalactia was first diagnosed in 1935 by Riegler and Stamatin (Manzat, 2001). The primary etiological agent of contagious agalactia in sheep and goats is *Mycoplasma agalactiae*. In goats, the disease can also be attributed to *Mycoplasma mycoides* subsp. *mycoides*, *Mycoplasma capricolum* subsp. *capricolum*, and *Mycoplasma putrefaciens* (Jaÿ & Tardy, 2019).

M. agalactiae is a small, polymorphic bacterium. The lack of cell was provides resistance to penicillin and its analogues, but the microorganism is susceptible to osmotic shock and the effect of detergents. Diagnosis through classical bacteriology is difficult to establish, as isolated strains adjust very slowly to laboratory conditions and may take over a week to develop colonies (Kumar et al., 2014).

The infection is often enzootic, causing mastitis in lactating female animals, with a consecutive drop or complete loss of milk production. It also affects non-lactating females, males and young animals, causing multiple clinical signs, such as pneumonia, arthritis, keratoconjunctivitis and sepsis (Madanat et al., 2001). Non-specific symptoms such as fever, anorexia and weakness can often be a cause of mortality in young animals, while going unnoticed in adult sheep and goats. Also, joint infections can be more severe in the young, taking the form of polyathritis, while in adults occasionally causing lameness (Jaÿ & Tardy, 2019). Primary sources of infection are diseased animals, which can spread the etiological agent through urine, feces and genital discharge. The disease can also be spread through infected milk. Animals that have overcome the disease can still remain carriers for up to 2 years (Manzat, 2001).

An outbreak of contagious aglactia can cause major economic loss to a herd, therefore, efforts to prevent the onset of the disease relay mainly on immunoprophylaxis. In Europe,

formalin inactivated vaccines against *M. agalactiae* are widely used. The vaccines produced using laboratory strains and usually contain an adjuvant such as aluminum hydroxide or an oil emulsion (OIE Terrestrial Manual, 2018). There have also been reports of phenol or saponin-inactivated *Mycoplasma* vaccines that were effective in experimental challenges (Tola et al., 1999).

The aim of this study was to determine and compare the efficacy of two contagious agalactia vaccines produced and marketed in Romania.

MATERIALS AND METHODS

The trials took place in Braila county, between November of 2019 and December of 2020. The animals included in the study belonged to a flock of over 1000 sheep. The flock included all categories of age and sex (rams, gestating females, lactating females, reformed females and lambs of both sexes). As per the producers' instructions, two categories of animals were omitted from the trial: lambs under the age of three months and female sheep during the last month of gestation. Also, only clinically healthy animals were selected. Also, the animals proving suspicious or positive serological results at day 0 were eliminated from the trial. The final number of animals included in the study was 703 subjects. For the immunization, two commercially available vaccines were selected (Vaccine A and Vaccine B), both Romanian products, by different producers. The composition of the two vaccines, as specified on each product's label, was as follows:

- Vaccine A: *Mycoplasma agalactiae* AG6 strain (≥0,60 ELISA units, according to the manufacturer data), inactivated with formalin (≤0.5 mg) and adsorbed onto aluminum hydroxide gel (2.8-3.4 mg Al_2O_3);
- Vaccine B: *Mycoplasma agalactiae* S/94 strain (minimum 5 ELISA units/dose, according to the manufacturer data), aluminum hydroxide gel (0.2-0.25 ml/1 ml of vaccine), formaldehyde (maximum 0.5 mg/ml).

Both vaccines are advertised to provide immunity against *M. agalactie* infection for 6 months.

The sheep were divided into three groups (Table 1). Group 1 (251 sheep) received two doses of Vaccine A, 21 days apart, via subcutaneous route, 1 ml/animal. Group 2 (249 sheep) was immunized with Vaccine B following the same protocol. Group 3 (203 sheep) represented the control group and was administered sterile saline solution via the same route.

Table 1. Trial design and group composition

Group/specification	Rams	Lambs > 3 months	Lactating females	Gestating females	Reformed females	Total
Group 1/Vaccine A	84	47	91	15	14	251
Group 2/Vaccine B	75	52	103	12	7	249
Group 3/Saline solution	52	38	76	24	13	203

The animals were monitored for 7 days after each inoculation in order to observe and record any systemic or local adverse reactions. Blood samples were collected from the animals on vaccination days (day 0 and day 21), and post vaccination, on days 30, 90, 180 and 360 (after the second dose of vaccine). The samples were collected using vacuum blood collection tubes coated with a clot activator. The tubes were kept at room temperature for four hours and were centrifuged 15 minutes at 2000 rpm, 4°C. The serum samples were collected into sterile Eppendorf tubes, identified with each animal's unique serial number and stored at -20°C until processing. The serological response was assessed by indirect enzyme-linked immunoassay (ELISA). A commercially available ELISA kit (CIVTEST ovis *M. agalactiae,* Hipra) was used according to the manufacturer's instructions. The mean antibody titers were calculated for each group, and also for each age and sex category inside the groups.

RESULTS AND DISCUSSIONS

Post-vaccination side effects included local reactions at the site of inoculation, less than 2 cm diameter, inapetence lasting 1-2 days after

vaccination, mainly in lambs, and a temporary (5-6 days) drop in milk production for the majority of the lactating ewes. The control group showed no general, nor local side effects, following the inoculation of the saline solution.

The calculation of the Rz values [Rz = OD_{450} Sample/2x (Mean OD_{450} Negative Control)] and interpretation of ELISA test results was performed according to the manufacturer's instructions (CIVTEST® ovis *M. agalactiae* Product Manual). All animals included in the study were free of antibodies against *M. agalactiae* at day 0 (the day of the first inoculation). The percentage of positive animals in Group 1 was slightly higher at day 21 then Group 2, with increased antibody titer means. Animals in both groups showed a significant rise in antibody titers 30 days after the second vaccination. At day 90, the serological response of both groups was still positive, with a slight decrease in antibody levels for the animals of Group 1. Testing at 180 days post-vaccination showed a marked decline in mean antibody titers for both groups, with only a small percentage of animals remaining positive. Very few positive results were recorded at the 360 days post-vaccination test.

The animals in the control group remained negative for the duration of the trial (Figure 1). The percentage of positive animals in each group and for each serological test is presented in Table 2.

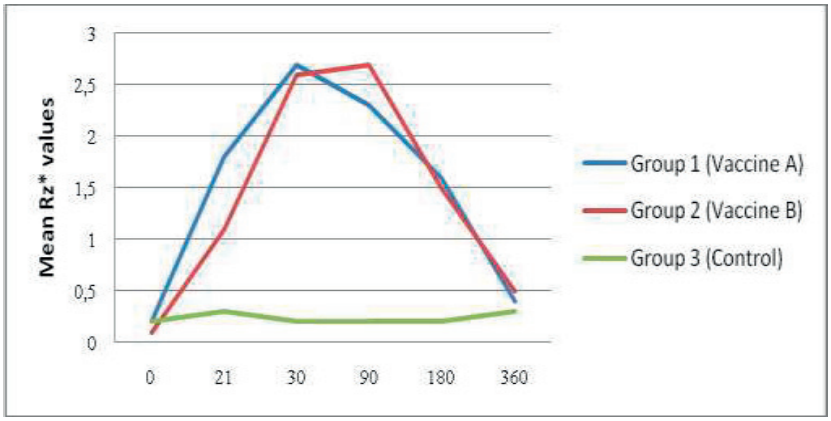

Figure 1. Mean Rz values throughout the trial period
(*Rz <1 - negative; Rz 1 - 1.5 - suspicious; Rz > 1.5 - positive)

It can be observed that even though antibody levels were higher in Group 1 at day 21, similar levels of protection were reached in both vaccinated groups 30 days after the booster. The immune response remained at close values for the next 2 months, with similar results obtained 90 from the booster. As expected, 6 months later, the mean Rz values dropped below the cut-off value of 1.5. It can be deduced from the test results that a repeating the vaccination scheme at a 6 months interval is necessary to ensure a constant level of protection in the flock. Mean antibody titers for each category of animals inside the vaccinated groups are shown in Figure 2 (Group 1 - Vaccine A) and Figure 3 (Group 2 - Vaccine B).

Table 2. Percentage of positive results for each test

Test Group	Positive animals (%)					
	Day 0	Day 21	Day 30	Day 90	Day 180	Day 360
Group 1 - Vaccine A	0	60	96	87	54	4
Group 2 - Vaccine B	0	45	94	96	58	11
Group 3 - Control	0	0	0	0	0	0

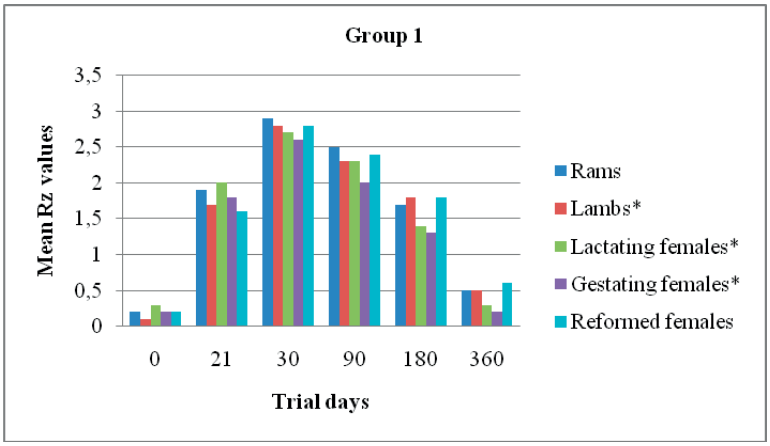

Figure 2. Evolution of antibody titers over the trial period for each category of animals in Group 1 (*the same animals were tested, even though their status changed over time)

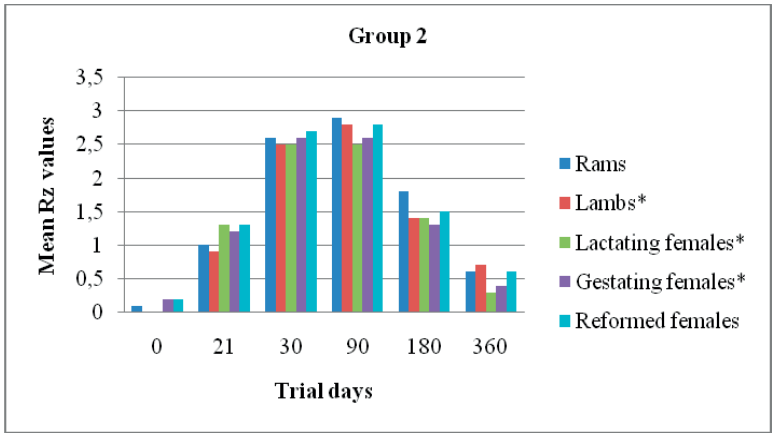

Figure 3. Evolution of antibody titers over the trial period for each category of animals in Group 2 (*the same animals were tested, even though their status changed over time)

The results presented in the study prove that both vaccines induced sero-conversion in the vaccinated animals of all ages and physiological status. For vaccine A, positive reactions were recorded after day 21. Antibody titers reached a maximum level at day 30 after the booster, and had started to decrease at day 90. At day 180, three of the five categories of sheep in Group 1 (rams, lambs and reformed females) still had positive reactions on the ELISA test.

For the animals in Group 2, the serological response of the vaccinated animals was below the cut-off value of 1.5 at day 21. At day 30, antibody titers had reached a similar level to those of Group 1, however, at day 90, the mean Rz values were higher. At day 180, antibody titers had decreased significantly, with only two categories of animals remaining positive on the ELISA test (rams and reformed females).

The efficacy of formalin inactivated vaccines against *M. agalactiae* has been investigated and disputed intensely for the last decades. In 2018, El-Yazid et al. studied the efficacy of four types of inactivated *M. agalactiae* vaccines, using formalin, phenol, saponin and sodium hypochlorite. The tests were carried out on mice and goats, and proved that the formalin inactivated vaccine provided only moderate protection in both serological and challenge trials. Saponin and phenol inactivated vaccines gave the highest level of protection, while the sodium hydroxide inactivated vaccine induced the lowest protective efficacy. Similar results

had been obtained before by researchers who demonstrated that phenol and saponin inactivated *Mycoplasma* vaccines induced the highest level of serological response in vaccinated sheep, compared to formalin, sodium hypochlorite and heat inactivated formulas (Tola et al., 1999). The level of immune response can also vary depending on the adjuvant used for vaccine preparation. A study carried out in Brazil reported superior results using Montanide IMS 2215 VG as adjuvant for a inactivated *Mycoplasma agalactiae* vaccine, compared to aluminum hydroxide and a Montanide Gel 01 adjuvanted vaccines (Campos et al., 2013). Other researchers have demonstrated that live attenuated vaccines provide superior clinical protection in sheep, despite the lack of serological response (Agnone et al., 2013; Ozdemir et al., 2019). However, live attenuated vaccines for contagious agalactia are not permitted in the European Union (OIE Terrestrial Manual, 2018).

CONCLUSSIONS

Both vaccines tested in the current study provided adequate levels of immune response in the vaccinated animals.

Results of the ELISA tests demonstrate that antibody levels decrease 6 months after vaccination, two vaccinations per year are necessary in order to provide a constant level of protection in the flock.

Further studies are necessary to assess the correlation between the serological response and the clinical protection provided by these vaccines.

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