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COMPARATIVE ANALYSIS OF IMMUNE RESPONSE IN GOATS AND SHEEP AFTER VACCINATION AGAINST CONTAGIOUS AGALACTIA – A FIELD STUDY

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ABSTRACT

The aim of the present study was to detect differences in immune response in sheep and goats after vaccination with a commercial vaccine against contagious agalactia. Serum samples from the two small ruminant species, reared in a mixed flock were collected on the day of vaccination and at post vaccination days 3, 7, 14, 30, 60, 90, 120, 180 and assayed by means of indirect ELISA. It was found out that antibody titre increased rapidly after application of vaccine, attained a maximum between the 7th and 30th day and rapidly declined after the 90th experimental day. Although the titres in sheep were higher than those in goats, no statistically significant difference was present.

Key words: *Mycoplasma agalactia*, Contagious agalactia, Goats, Vaccines, ELISA.

Introduction

In lactating animals it is manifested with mastitis with subsequent decline in milk yield and agalactia. Concomitant clinical signs are arthritis and keratoconjunctivitis, sometimes septicaemia in young animals.

Contagious agalactia is an infectious disease of sheep and goats caused mainly by *Mycoplasma agalactiae* (*M. agalactiae*). In lactating animals it is manifested with mastitis with subsequent decline in milk yield and agalactia. Concomitant clinical signs are arthritis and keratoconjunctivitis, sometimes septicaemia in young animals. A disease with similar clinical signs, often accompanied with respiratory signs could be induced by *Mycoplasma capricolum* subsp. *capricolum, Mycoplasma mycoides* subsp. *mycoides*, *Mycoplasma mycoides* subsp. *capri* and *Mycoplasma putrefaciens* (Da Massa et al., 1992; Bergonier et al., 1997; Nicholas, 2002). The organism formerly known as *M. mycoides* subsp. *mycoides* large colony (LC) type has been incorporated into *M. mycoides* subsp. *capri* (Manso-Silvan et al., 2009). The disease is encountered at a global scale and is common in high milkproducing regions. Most Mediterranean countries are considered endemic (Corrales et al., 2007). The disease incurs substantial 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 issued by the World Organisation for Animal Health (OIE).

The infection spreads rapidly affecting 30–85% in a short time period. The antibiotic treatment for 5 to 10 days reduces the main clinical signs of disease, but does not eliminate carriership of bacteria (Azevedo et al., 2006). The control of mycoplasmosis with antibiotics is of little value, yet it turns the clinically recovered animal into a carrier.

Applied preventive measures are not always efficient enough due to insufficient knowledge of disease epidemiology, specific features related to sheep and goats reactivity and availability of efficient vaccines against the various etiological agents. In Europe, vaccination is practice since the 1970s (Foggie et al., 1970) but its extensive application dates back after the 1990s. Inactivated monovaccines against *M. agalactiae* are mainly used, or combinations with *M. mycoides subsp*. *mycoides* LC, *M. putrefaciens*, *M. capricolum* subsp. *capricolum*. The real efficacy of this vaccine however is questionable, especially under field conditions (Nicholas, 1995; Bergonier et al., 1997).

There are several reports about lack of efficient protection after application of standard inactivated vaccines in goat flocks (Villalba et al., 1991). This was attributed to the high degree of antigenic variability, observed in field *M. agalactiae* strains (Solsona et al., 1996; Tola et al., 1996; De la Fe et al., 2006).

The present study aimed to evaluate the compare the immune response in goats and sheep treated with a commercial vaccine against contagious agalactia in field conditions.

Materials and methods

Flock

The field study was carried out in a flock with 65 animals – 55 sheep and 10 goats. Both species were from a mixed breeds. They were reared in a stable with indoor and outdoor sections. Animals were fed alfalfa hay, concentrate and grazed on pasture. Prophylactic treatments against helminths were performed twice each year. At 6-month intervals, upon owner's decision, they were vaccinated against contagious agalactia. Eight sheep and 8 goats, 2-5 years of age with history of previous vaccinations were randomly selected. None of management conditions applied to the flock prior to the trial has been changed during the experiments. Throughout the trial, no antibiotic therapy neither another specific treatment against *Mycoplasma* was applied.

Vaccine

A commercial phenol-inactivated vaccine Agalax-S with aluminium hydroxide as adjuvant (Laboratorio SYVA) was used, stored under conditions stipulated by the manufacturer and applied at a dose as per manufacturer's recommendations.

Study design

The one-year experimental period was divided into 2 six-months subperiods as per vaccination schedule against contagious agalactia approved in the farm. The first period was from November 2018 to April 2019, and the second one: from May to October 2019.

Jugular venous blood samples were collected from all experimental animals at the day of vaccination (day 0), and post vaccination days 3, 7, 14, 30, 60, 90, 120, 180. Day 0 of the second 6-month period was the 180th day of the first period. Plain blood samples were collected in vacutainers. Sera were separately collected by centrifugation and kept at –20°C until use.

ELISA

Samples were assayed with commercial indirect ELISA kit – ID Screen Indirect (IDvet, 310, rue Louis Pasteur – Grabels – France). This kit was designed for detection of antibodies against *Mycoplasma agalactiae*, causing contagious agalactia in small ruminants (sheep, goats).

The wells of plates are loaded with purified *M. agalactiae* P48 precombinant proteins, used as target antigen. If samples contained anti-P48 antibodies, a antigen-antibody complex was formed and fixed to the well bottom by adding a conjugate – horseradish peroxidase (HRP). The conjugate's

enzyme formed an antigen-antibody-conjugate-HRP complex. After washing of wells for removal of excess conjugate, a substrate solution was added to visualize the reactions (coloration in blue). In the presence of antibodies, the blue colour became yellow after addition of stop solution.

Microplate was read at 450 nm with ELISA reader LEDETEC LABEXIM PRODUKTS.

The results were interpreted by calculated S/P percentage for each sample using the following formula:

$$S/P\% = \frac{\text{OD of sample} - \text{OD of negative control (NC)}}{\text{OD of positive control (PC)} - \text{OD NC}} \times 100$$

Samples with S/P% ≤ 50% were considered negative, samples with S/P% > 50% and < 60% were interpreted as doubtful, and samples with S/P% ≥ 60% were considered positive.

Statistical analysis

Data were analyzed with GraphPad Prism 7.04 (GraphPad Software Inc., La Jolla, CA) by one-way ANOVA to determine Mean (x), standard error of mean (SEM) values, and the statistically significant differences in mean values of all data. $P < 0.05$ was considered statistically significant.

Results

The results from serological tests of goats showed a gradual increase in antibody titres up to post vaccination day 14, followed by a reduction. S/P% values close to baseline ones (80.42 ± 26.44) were attained on post vaccination day 120 (96.84 ± 19.3). By the 180th day of the experimental period, S/P% values (36.98 ± 13.91) were about twice lower that initial ones. There were no statistically significant differences between values throughout the period.

During the second 6-month period, despite the lower initial values, antibody titres increased sharply after the vaccination and peak was reached again on the 14th day. This increase was substantially higher between day 0 vs post vaccination days 7 and 14 ($P = 0.0483$ and $P = 0.0309$ respectively) and between day 3 vs days 7 and 14 ($P = 0.0399$ and $P= 0.0221$ respectively). Afterwards, a decline was noted with values close to baseline ones by the 90th day (80.5 ± 14.7) of the second period.

The analysis of sheep samples demonstrated similar tendencies in the time course of antibody titres – increase during the first post vaccination weeks followed by gradual decrease and attainment of initial levels about post vaccination days 90–120. In sheep, the differences were not statistically significant.

The summarised results for the two small ruminant species in the two experimental periods and presented in Table 1.

The comparison of post vaccinal immune response against contagious agalactia in goats showed a very comparable reaction in both periods with comparable maximum levels on the 14th day and slow decrease (Fig. 1).

Table 1: Kinetics of serological responses (ELISA test) in goats and sheep immunized with commercial vaccine against contagious agalactia.

	S/P%			
	Goats		Sheep	
	First period	Second period	First period	Second period
Day 0 Mean ± SEM	80.42 ± 26.44	36.98 ± 13.91	96.15 ± 18.01	88.54 ± 27.75
Day 3 Mean ± SEM	71.59 ± 24.64	44.23 ± 11.06	98.26 ± 20.89	80.13 ± 28.88
Day 7 Mean ± SEM	93.6 ± 12.14	113.9 ± 35.76*	141.1 ± 13.49	125.2 ± 34.76
Day 14 Mean ± SEM	108 ± 23.4	114.5 ± 29.14*	130.7 ± 10.28	129.4 ± 25.07
Day 30 Mean ± SEM	118.7 ± 17.55	103.5 ± 19.18	126.2 ± 10.8	112.7 ± 23.92
Day 60 Mean ± SEM	141.3 ± 25.94	90.91 ± 16.72	111.9 ± 9.954	111.0 ± 18.72
Day 90 Mean ± SEM	106.2 ± 21.67	80.5 ± 14.7	98.17 ± 9.794	105.1 ± 20.87
Day 120 Mean ± SEM	96.84 ± 19.3	62.78 ± 14.01	88.4 ± 8.922	82.31 ± 19.32
Day 180 Mean ± SEM	36.98 ± 13.91	57.35 ± 13.26	88.54 ± 27.75	68.0 ± 13.51

** $P < 0.05$*

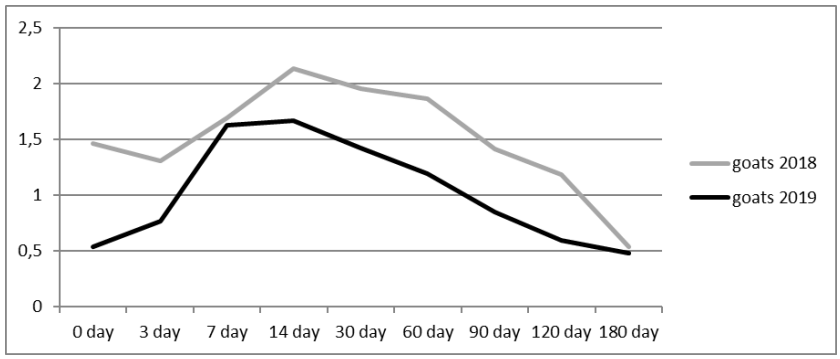

Figure 1: Time course of IgG antibody titres (OD value) against *M. agalactiae* in goats.

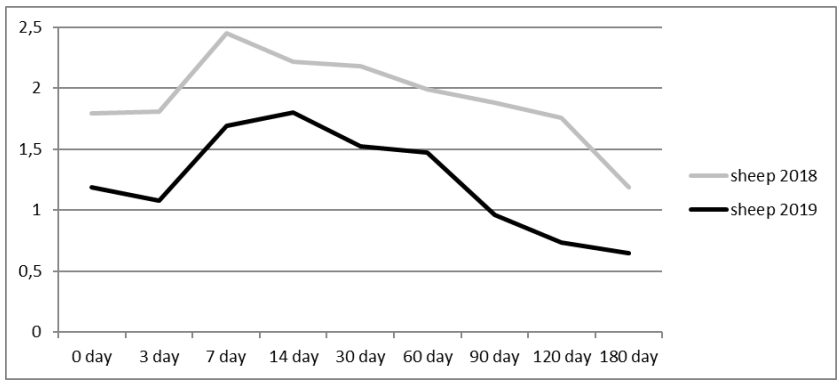

Figure 2: Time course of IgG antibody titres (OD value) against *M. agalactiae* in sheep.

In sheep, antibody titre dynamics was similar to that observed in goats, but the peak levels during the first period were attained as early as post vaccination day 7 without statistically significant differences from those during the same day of the second period (Fig. 2).

The comparison of data for both small ruminant species showed that sheep OD values were statistically insignificantly higher. Although the initial values in goats in the second period were lower, as early as post vaccination day 7, attained values were very close to those in sheep and this trend was preserved until the end of the experiment (Fig. 3).

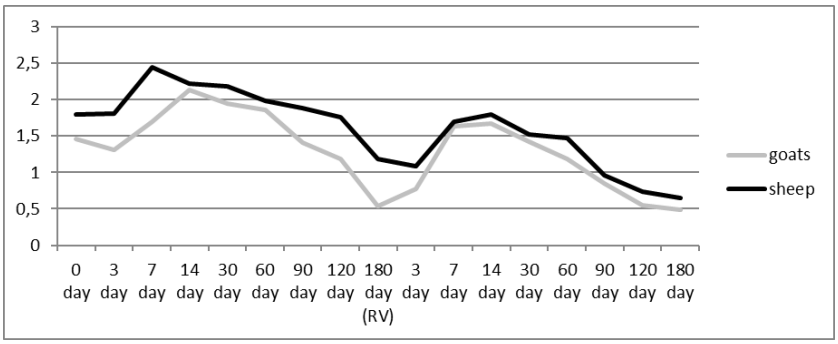

Figure 3: Comparison of immune response (OD value) of sheep and goats against *M. agalactiae*.

Discussion

Vaccines are designed to stimulate immune response against a pathogen, so that a future contact with it would not provoke a clinical disease. The use of vaccines for control of contagious agalactia in endemic regions is a common practice. Most available data on the efficacy of existing vaccines are obtained under experimental conditions. Literature data for the effect of commercial products in the field are scarce. Even fewer data referring to comparative evaluation of immune reactivity in sheep and goats vaccinated against contagious agalactia are available.

In the present study, the antibody titre increased rapidly during the first two post vaccination weeks and declined progressively to reach initial levels on the third month. Similar results were presented by Erdag (1989) in tests of inactivated vaccine in Turkey. IN this study, antibodies detected by complement fixation and growth inhibition tests appeared 2 weeks after the vaccination, attained a peak 4 weeks alter and disappeared 10 weeks following vaccination.

Tola et al (1999) used ELISA to detect antibodies in vaccinated sheep after administration of various inactivated vaccines. The authors vaccinated the animals before breeding and during the gestation and reported that saponin and phenol inactivated vaccines maintained high levels of antibodies with two peaks: one during the 3rd month and another, 8 months after the vaccination.

Results from the present study were comparable – we found out a peak by the first post vaccination month and another one during the 7th month, attributed to performed revaccination.

In a more recent study having tested several experimental vaccines, El-Yazid et al (2019) demonstrated antibody peak in phenol-inactivated vaccine containing aluminium hydroxide by the 8th week after its application – a result, different from ours. The difference is due to the fact that the cited study used animals that were not previously vaccinated, whereas the small ruminants used in the present study had a history of several previous vaccinations.

Comparative studies on the immune response in sheep and goats were carried out by Sotoodehnia et al (2005). They investigated an own inactivated vaccine against contagious agalactia

in small ruminants. Vaccination schedule included two applications of the product at 3-week interval. The authors showed maximum antibody levels one month after the first vaccination (1 week following revaccination), minimum average titres by the 3rd month respectively: results, that were completely identical to ours. At the same time, the authors observed lower values in goats as compared to sheep. The difference in antibody response in sheep and goats was attributed to the origin of the strain, which was isolated from sheep.

Similarly, to Sotoodehnia et al (2005), Campos et al (2013) have tested several types of inactivated vaccines. However, they established slightly higher antibody levels in goats than in sheep. Although the values of optical density have been slightly higher in goats compared to sheep, there was no significant statistical difference between both species ($P>0.05$) for formalin-inactivated vaccine with aluminium hydroxide as adjuvant. Similarly, to the present study, OD values in sheep and goats in the experiments of Campos et al., (2013) declined progressively to attain baseline values 3-4 months after the beginning of the trial.

In the present study, initial antibody titres in sheep and goats exceeded those found on post vaccination month 6. Regardless of the sharp increase following revaccination, OD values did not reach the peaks from the preceding period. This could be attributed to the fact that revaccination was done in the spring in postpartum animals that have nursed their offspring and were still lactating. All this leads to body depletion and weaker post vaccinal reactivity.

In our study, lower but statistically insignificant differences in antibody titres against contagious agalactia vaccine were found out between sheep and goats. At the same time, a very clear tendency in S/P% reduction by the 3rd- 4th month post vaccination was evident. In the first period of the study, the values were < 50 by the 180th day, which was interpreted as negative result, presuming a lack of protection following infection in the field. In sheep, S/P% remained higher than 60 throughout the entire study period. The difference in the reactivity of both small ruminant species was probably due to the vaccinal strain which was of ovine origin; a hypothesis expressed also by Sotoodehnia et al (2005). The result demonstrated that the vaccination schedule for this flock should be corrected and despite the opposition from the part of the owners, prophylactic vaccinations should be done at 4-month intervals. Similar recommendations were made by Regalla (1987), who advised immunisation three times per year regardless of considering vaccination as inconsistent and non-satisfactory when animals inhabited an environment with very high level of *Mycoplasma* contamination. León et al (1995) also recommended application of three doses of the vaccine before and one dose after the parturition for control of the disease.

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