HUMORAL IMMUNE RESPONSE IN LAMBS AND GOAT KIDS INOCULATED WITH A DUAL VACCINE AGAINST CONTAGIOUS AGALACTIA

Respuesta inmune humoral en corderos y cabritos inoculados con una vacuna bivalente frente a agalaxia contagiosa

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ABSTRACT

A combined vaccine against *Mycoplasma agalactiae* and *Mycoplasma mycoides* subsp. *mycoides* (large colony type) was developed using inactivated strains selected in previous characterization studies. Phenol was used as inactivating agent and aluminium hydroxide was added as adjuvant. The present study was designed to evaluate the specific humoral immune response to these two mycoplasma species shown by lambs and goat kids. One group of 15 Pelibuey lambs and one group of 8 Canary goat kids received two injections of vaccine at the ages 45 and 70 days and were monitored until they were 120 days old. Antibody titres were determined by indirect ELISA. Lambs showed lower antibody levels (expressed as ODs) to both antigens than goat kids althought significantly differences were only registered to *M. agalactiae* antigen ($P<0.005$).

Key words: contagious agalactia / combined vaccine / humoral immune response / lambs / goat kids

RESUMEN

Una vacuna polivalente frente a *Mycoplasma agalactiae* and *Mycoplasma mycoides* subsp. *mycoides* (large colony) fue elaborada utilizando cepas inactivadas seleccionadas en base a previos estudios de caracterización de las mismas. El fenol se utilizó como inactivante mientras que como adyuvante se utilizó el hidróxido de aluminio. El trabajo evaluó la respuesta immune humoral inducida en un grupo de 15 corderos Pelibuey y 8 cabritos de la raza canaria majorera, los cuales recibieron 2 dosis de vacuna a los 45 y 70 días de edad, siendo monitorizados hasta los 4 meses de vida. Los títulos de anticuerpos observados por ELISA indirecto muestran

títulos menores en el grupo de corderos que en el grupo de cabritos frente a ambos antígenos, siendo estas diferencias estadísticamente significativas sólo frente a *M. agalactiae*.

Palabras clave: agalaxia contagiosa, vacuna combinada, respuesta inmune humoral, corderos y cabritos

INTRODUCTION

Mycoplasma agalactiae (*M. agalactiae*) and *Mycoplasma mycoides* subsp. *mycoides large-colony type* (LC) (*Mmm* LC) are the main causative agents of contagious agalactia (CA) syndrome in sheep and goat. This diseases, which is characterised by agalactia, mastitis, arthritis, keratoconjunctivitis, and sometimes abortion and pneumonia, is present all over the world with a remarkable importance around the Mediterranean basin (Nicholas, 1995).

Several preventive strategies have attempted to minimize contagious agalactia clinical expressions, including vaccination. In this sense, numerous inactivated vaccines (mainly against *M. agalactiae*) have been developed and applied to control the syndrome but no single vaccines have been universally adopted and no standard methods of preparation and evaluation has been applied (OIE, 2006). These have also raised several concerns related to efficacy, especially at field conditions (Bergonier et al., 1997). In this sense, several failed vaccines may be attributed to the high degree of antigenic variability observed in field strains of *M. agalactiae* and *Mmm* LC (Solsona et al., 1996; Tola et al., 1996; De la Fe et al., 2006[a]) or to the aetiological variation observed in goat herds (OIE, 2006). For this reason, the use of combined vaccines in endemic areas has been proposed to control the disease (Consenti and Montagna, 1989). Recently, a set of dual vaccines against *M. agalactiae* and *Mmm* LC were developed in Spain, using inactivated strains selected in previous characterization studies (De la Fe et al., 2006[a]). Later, diverse test were realized under field conditions (De la Fe et al., 2006[b]; De la Fe et al., 2007). This study, a part of these experiments, was designed to evaluate the humoral immune response in lambs and goat kids injected with a similar dual vaccine against these two mycoplasma species.

MATERIALS AND METHODS

Vaccine tested was prepared using killed field strains of both mycoplasma species selected according to their previously published protein and antigenic profiles (De la Fe et al., 2006[a]). Field strains used were *M. agalactiae* (L9, AGIN3, 9B) and Mmm LC (AG1, 153/93, IN3) isolated from local clinical cases of caprine CA. The vaccines were prepared using cells of these strains cultured in modified PH culture medium (Kirchhoff and Rosengarten, 1984) supplemented with 10% horse serum (BioWhitaker, USA) at 37°C until the mid-log phase. The cells were then centrifuged at 20,000×g for 30 min at 4ºC and resuspended in saline (0.9% NaCl; pH 7.2); this process was repeated three times. Colony forming units (CFU)/ml (Albers and Fletcher, 1982) were determined and each 2-ml dose was prepared to contain more than 5 x 10^{10} cfu/ml of each mycoplasma species. Washed cultures containing pooled mycoplasma were stored at -20°C. Aluminium hydroxide (25%, vol/vol, Superfos A/S, Vedbaek, Denmarck) was added as adjuvant, and the vaccine then incubated with constant rocking for 2 h at 37°C. Vaccine was inactivated with phenol (0.5%, vol/vol, Sigma-Aldrich, Madrid, Spain, for 16 h at 37°C) and stored at 4ºC. The side effects of the vaccines were determined by intraperitoneal administration of 1 ml of each vaccine to 10 mice and by subcutaneous inoculation of 10 ml of the vaccines in two goats.

Study was carried out at the Veterinary Faculty of Las Palmas University (ULPGC), Gran Canaria, Spain. One group of 8

Table 1: **Serological results obtained in both vaccinated groups. The mean of the optical densities (OD) and the standard deviation is indicated in each case**

Day	*M. agalactiae* Mean (standard deviation)		*Mmm LC* Mean (standard deviation)	
	Lambs	Goat kids	Lambs	Goat kids
45	0,238 (0,006)	0,211 (0,008)	0,265 (0,008)	0,220 (0,011)
70	0,468 (0,018)	0,561 (0,025)	0,533 (0,026)	0,613 (0,036)
120	0,396 (0,018)	0,457 (0,024)	0,492 (0,025)	0,573 (0,034)

seronegative Canary goat kids and one group of 15 seronegative Pelibuey lambs were selected. Both groups received two subcutaneous injections (2 ml of each) at the ages 45 and 70 days. The animals were monitored until they were 120 days old, and blood samples withdrawn from all the animals at the ages 45, 70 and 120 days. Sera were immediately prepared from each blood sample and kept at -20ºC at the ULPGC. Anti-*M. agalactiae* and anti-Mmm LC antibody levels, expressed as optical densities (OD), were determined by an ELISA procedure previously described (Assunçao et al., 2004). For the preparation of the *M. agalactiae* antigen the local strains of mycoplasma designed as L9, 21 Pi and Pla 4 were used, and for the *Mmm* LC antigen the strains 207/93, 153/93 and 2/93 were used. The antigens were prepared as described by Nicolet and Paroz (1980) and Bereiter et al. (1990). To establish cut-off values and sensitivity and specificity of the assays, 108 sera derived from experimentally inoculated animals and 100 negative sera were used. The sera were considered positive for *M. agalactiae* and *Mmm* LC if the OD were greater than 0.300 and 0.350, respectively. This method shows a sensitivity of 99 per cent and a specificity of 78 per cent for *M. agalactiae* and a sensitivity of 100 per cent and a specificity of 84 per cent for *Mmm* LC. To statistically analyse the results obtained, we considered the effects of the animal species, observation day, the interaction of both these effects, the random effect of the experimental unit and the residual effect, which were all assumed to be normally distributed. The model was estimated by the method of maximum likelihood. The significance of the effects was tested by the corresponding *F*-Test. When the treatment effect was found to be significant, Scheffe's multiple comparisons were performed. The level of statistical significance was set at $p < 0.05$. All statistical tests were performed using SPSS software (version 11.5) for Windows.

RESULTS

Sterility tests were negative. None of the vaccinated mice died and the goats that received two doses of the vaccines showed no reaction after vaccination. During the study, none of the vaccinated animals showed reaction after vaccination.

Serological results indicate a steady increase in the production of specific IgG antibodies against *M. agalactiae* and *Mmm* LC in both groups of vaccinated animals (Table 1). Moreover, lambs showed significantly lower antibody levels (expressed as ODs) to *M. agalactiae* antigen than goat kids (P<0.005) at 70 and 120 days. In both groups, serum IgG levels peaked in the third month (70 days). On Day 120, the antibody levels shown a slightly decrease in both vaccinated groups.

DISCUSSION

Findings registered in previous field trials realized with this type of dual vaccines indicate the effectiveness of the vaccines in preventing the appearance of new clinical signs such as mastitis, abortion, pneumonia and polyarthritis in CCA affected herds (De la Fe et al., 2007) or revealed the production of specific antibodies against these two mycoplasma species in vaccinated lactating goats (De la Fe et al., 2006[b]).

The present study was realized to compare the humoral immune response induced in goat kids and lambs by these dual vaccines. In both ruminant species, antibodies peak were registered at a similar time to when a monovalent *M. agalactiae* vaccine is administered (Foggie et al., 1971[a]; Buonavoglia et al., 1998) although lambs showed lower antibody levels (expressed as ODs) to both antigens than goat kids. Significantly differences were only registered against *M. agalactiae* antigen. No information is available about the possible differences in the humoral immune response of both ruminant species against mycoplasmas involved in CA. Only Foggie et al. (1971[a], 1971[b]) registered a better immune response in sheep than goat after the inoculation of a *M. agalactiae* vaccine, although no information is showed about the origin of the strain used to elaborate it. Our results could be explained by the use of vaccines elaborated with mycoplasma strains isolated from goats, although further studies are necessaries in order to improve the knowledge about this fact. In this sense, no information is also available about protein or antigenic differences of *M. agalactiae* strains from goat or sheep respectively. In this case, it could be suitable the use of specific-species strains isolated from goat or sheep to elaborate their respective vaccines.

All these trials represent the first report of the use of combined CA vaccines prepared using killed field strains of mycoplasma selected according to their previously published protein and antigenic profiles (De la Fe et al., 2006[a]).

This new strategy could produce a more efficient CA vaccine since reference strains lack many of the antigens shown by field strains (Gummelt et al., 1996), and the use of a pool of field strains could reduce the number of vaccines that fail because of the antigenic variability of *M. agalactiae* and *Mmm* LC.

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