World Rabbit Sci. 2003, 11: 227 - 232

TECHNICAL NOTE

AUTOVACCINES AGAINST MYCOPLASMAS IN FARM RABBITS

BOUCHER S*., NOUAILLE L*., ALBIZU I†., BASELGA R†.

*Cabinet Veterinaire SCP NSBABGVC, Zac de la Buzeniere
BP 539, 85505 LES HERBIERS, France.

†Exopol. Polig. Río Gállego, San Mateo de Gállego,
50840 ZARAGOZA, Spain.

ABSTRACT: Farm rabbits are affected by respiratory and reproductive disorders that compromise their health and the productivity of the farm. In two earlier papers we highlighted the role of mycoplasmas in these processes and the present article evaluates the usefulness of autovaccines to control mycoplasmosis. On a commercial rabbitry with 800 females, 428 kits were vaccinated, leaving 3,622 as controls. A lower mortality was observed among the vaccinated kits, with a significant ($P<0.01$) decrease in the presence of lung lesions.

Key words: rabbit, mycoplasma, autovaccines.

INTRODUCTION

Farm rabbits are affected by respiratory and reproductive disorders (BOUCHER and NOUAILLE, 2002), which compromise health and productivity of the farm. In two earlier papers we highlighted the role of mycoplasmas in these processes, in a similar way to the observations in rodent species (BROWN and REYES 1991; FURR *et al.*, 1994). The use of an immunoperoxidase technique allowed the detection of *Mycoplasma pulmonis* in 43.1 % of the lungs of animals with respiratory problems,

Correspondence: R. Baselga.
E-mail: rbaselga@exopol.com

and in 38% of the genital tracts of females with reproductive problems, while it was only detected in 3% and 0% of healthy animals, respectively (BOUCHER *et al.*, 2001; VILLA *et al.*, 2001).

In the samples received in our laboratory (Exopol) we detected mycoplasmas in some samples in 42% of the 98 cases received with reproductive problems, and in 48% of the 333 cases with respiratory problems in which the veterinary surgeon requested the evaluation of mycoplasmas (data not shown).

Some mycoplasma species have revealed the presence of a capsular exopolysaccharide layer (CPS) enveloping the cells (ALMEIDA AND ROSENBUSCH 1991; RURANGIRWA *et al.*, 1995; BANSAL *et al.*, 1995; NIANG *et al.*, 1998), and although no investigations have been made on its effect, we may presume that CPS constitutes a virulence factor acting in the same way as in certain bacterial species, contributing to the avoidance of phagocytosis by the host macrophages and neutrophils (BOYCE AND ADLER, 2000).

In order to phagocytose encapsulated bacteria, the host must produce antibodies against CPS, which isn't very immunogenic. CPS can be immune-enhanced by covalently binding it to transporter proteins, as is done with *Haemophilus influenzae* in humans and as is also being investigated for *Streptococcus pneumoniae* (AHMAD AND CHAPNICK, 1999; PELTOLA, 1999) and other bacteria such as *Staphylococcus aureus* (FATTOM AND NASO, 1997). Immune enhancement can also be achieved using liposomes to vehicle antigen (AMORENA *et al.*, 1994; WONG *et al.*, 1992; GARCON AND SIX, 1991).

M. pulmonis is a very serious pathogen in laboratory mice and rats, and considerable effort has focused on the development of a vaccine to control the disease (LAI *et al.*, 1995). The present study describes the use of liposomes and exopolysaccharides of mycoplasmas in the preparation of autovaccines for the protection of rabbits on commercial farms.

MATERIAL AND METHODS

Description of the farm

The study was carried out on a farm in Mouchamps, France. This was a closed-cycle farm with a capacity of 800 females, which had had a chronic pneumonia problem for a number of years.

Vaccination was carried out in two maternities: one semi-intensive and the other closed, as reflected in Table 1. Kits were later fattened (32 days) on a semi-intensive facility independent of the maternity areas.

Strain

The mycoplasma strain used was isolated from lung tissue of animals with respiratory problems from the farm in which the autovaccine was evaluated. The strain was isolated as previously described (VILLA *et al.*, 2001).

Exopolysaccharide purification and preparation of autovaccine

The exopolysaccharides were essentially purified as previously described (AMORENA *et al.*, 1994), though the mycoplasma strain growth times were prolonged for 5-6 days in mycoplasma broth supplemented with porcine serum (20%).

Immune enhancement of the exopolysaccharides was based on the use of liposomes elaborated according to the "dry reconstituted liposomes" technique, as previously described (AMORENA *et al.*, 1994). Commercial aluminum hydroxide was used for resuspension of the lyophilized liposomes ($AlOH_3$; Rehydragel®, Reheis, Dublin).

Vaccination procedure

As described by BOUCHER AND NOUAILLE (2002), the animals were vaccinated on days 1 and 18 of life with 0.5 ml of the autovaccine. A total of 428 kits were vaccinated, leaving 3,622 kits as controls. The vaccinated group was chosen by randomized selection of the females at the time of vaccination, and the entire litter

was vaccinated, without sex distinction.

Statistical analysis

Results were analyzed by a chi-square test.

Clinical evaluation

All the losses were recorded. The lung lesions were examined among the losses recorded, as well as in some 3-4 weeks old animals randomly selected by an investigator without regard to the group to which each animal belonged.

RESULTS AND DISCUSSION

In human medicine, systematic use is made of vaccines against bacterial meningitis exclusively based on bacterial CPS. However, CPS is very scantly immunogenic, as a result of which some of these vaccines involve covalent binding of CPS to transporter proteins. However, this solution is not feasible in veterinary practice because of its high cost.

Liposomes have been used as vehicles for many different drugs, as well as T-dependent antigens (GREGORIADIS *et al.*, 1999) and, as in this case, T-independent antigens (GARCON AND SIX, 1991; PIETROBON *et al.*, 1994). Moreover, they have been used successfully in field tests against *Streptococcus suis* (MORILLO *et al.*, 2002) and experimental infections with *Staphylococcus aureus* (AMORENA *et al.*, 1994). Liposome preparation is very simple and inexpensive, and the use of lyophilized liposomes facilitates the storage and transport of the prepared autovaccines.

In other species such as swine, for example, mycoplasma infects neonates in the first week of life, and vaccinations take place in very early stages of life. Our first experiences (data not shown) also underscored the advisability of early vaccination in rabbits; for this reason 1 and 18 days old animals were selected.

Table 1: Mortality and frequency of lung lesions.

	Vaccinated		Non vaccinated	
	Closed maternity	Semi-intensive maternity	Closed maternity	Semi-intensive maternity
Post weaning	251	177	1732	1890
% Mortality	4.4	3.9	6.1	6.1
Autopsy of dead animals/ animals with pulmonary lesions	11/2	7/0	89/77	76/51
Slaughtered at lot end/ animals with pulmonary lesions	10/1	10/2	5/5	5/5

The results obtained with this vaccination protocol show that although the mortality losses were not significantly lower as a result of vaccination, the percentage of lung lesions found in necropsied and sacrificed animals was much lower among the vaccinated ones ($P<0.001$; Table 1).

REFERENCES

AHMAD H., CHAPNICK EK. 1999. Conjugated polysaccharide vaccines. *Infect Dis Clin North Am; 13: 113-133.*

ALMEIDA R.A., ROSENBUSCH R.F. 1991. Capsulelike surface material of *Mycoplasma dispar* induced by in vitro growth in culture with bovine cells is antigenically related to similar structures in vivo., *Infect. Immun. 59, 3119-3125.*

AMORENA B., BASELGA R., ALBIZU I. 1994. Use of liposome immunopotentiated exopolysaccharide as a component of an ovine mastitis staphylococcal vaccine. *Vaccine 12, 243-249.*

BANSAL P., ADEGBOYE D.S., ROSENBUSCH R.F. 1995. Immune responses to the capsular polysaccharide of *Mycoplasma dispar* in calves and mice. *Comp. Immunol. Microbiol. Infect. Dis. 18, 259-268.*

BOUCHER S., NOUAILLE L. 2002. Les Mycoplasmoses. *in: BOUCHER S., NOUALILLE L (ed Maladies des Lapins, Edition France Agricole, 2. Edition. 84-87.*

BOUCHER S., GRACIA E., VILLA A., FERNANDEZ A., NOUAILLE L., BRIFFAUD M.A. 2001. Pathogens in the reproductive tract of farm rabbits. *Vet. Rec. 149, 677-678.*

BROWN M.B., REYES L. 1991. Immunoglobulin class- and subclass-specific responses to *Mycoplasma pulmonis* in sera and secretions of naturally infected Sprague-Dawley female rats. *Infect. Immun. 59, 2181-2185.*

BOYCE H,D,M ADLER B. 2000, The capsule is a virulence determinant in the pathogenesis of *Pasteurella multocida. Infect Immun 68, 3463-3468*

FATTOM A.I., NASO R. 1997. Vaccines for *Staphylococcus aureus* infections. New Generation Vaccines, Ed. M. Levine et al. Marcel Dekker Inc. New York; Chapter 62.

FURR P.M., SARATHCHANDRA P., HETHERINGTON C.M., TAYLOR-ROBINSON D. 1994. Site of localization of *Mycoplasmas pulmonis* and *Mycoplasma hominis* in the genital tract of female mice demonstrated by culture and scanning and immuno-electron microscopy. *Int. J. Exp. Pathol. 76, 131-143.*

GARCON N.M., SIX H.R. 1991. Universal vaccine carrier. Liposomes that provide T-dependent help to weak antigens. *J. Immunol. 146, 3697-3702.*

GREGORIADIS G., MCCORMACK B., OBRENOVIC M., Saffie R., Zadi B., Perrie Y. 1999. Vaccine entrapment in liposomes. *Methods. 19, 156-162.*

LAI W.C., BENNETT, M., PAKES, S.P., MURPHREE, S.S. 1995. Potential subunit vaccine against *Mycoplasma pulmonis* purified by a protective monoclonal antibody. *Vaccine 9, 177-184*

MORILLO A., GASCÓN A., ALBIZU I., BASELGA R. 2002. Evaluación de autovacunas frente a *Streptococcus suis* basadas en exopolisacáridos capsulars. *Anaporc. 228, 1-7.*

Niang M., ROSENBUSCH R.F., ANDREWS J.J., KAEBERLE M.L. 1998. Demonstration of a capsule on *Mycoplasma ovipneumoniae. Am. J. Vet. Res. 59, 557-562.*

PELTOLA H. 1999. Prophylaxis of bacterial meningitis. *Infect. Dis. Clin. North. Am. 13, 685-710.*

PIETROBON P.J.F. GARCON N.M., LEE C.H., Six H.R. 1994. Liposomes that provide T-dependent help to weak antigens (T-independent antigens). *Immunomethods. 4, 236-243.*

RURANGIRVA F.R., WAMBUGU A., KIHARA S.M., MCGUIRE T.C. 1995. A *Mycoplasma* strain F38 growth-inhibiting monoclonal antibody (WM-25) identifies an epitope on a surface-exposed polysaccharide antigen. *Infect. Immun. 63, 1415-1420.*

VILLA A., GRACIA E., Fernandez A., ALBIZU I., BASELGA R. 2001. The detection of mycoplasmas in the lung of rabbits with respiratory disease. *Vet. Rec. 23, 788-789.*

WONG J.P., CHERWONOGRODZKY J.W., DINNO V.L., STADNYK L.L., KNODEL M.H., DININNO V.L. 1992. Liposome potentiation of humoral immune response to lipopolysaccharide and O-polysaccharide antigens of *Brucella abortus. Immunology. 77, 123-128.*