

## Subcutaneous and conjunctival vaccination with a live attenuated strain of *Salmonella Abortusovis*: effect of gestation on serological response of ewes

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**Summary** — Annual serological testing of flocks vaccinated by the subcutaneous route with a live, attenuated strain of *Salmonella Abortusovis* has previously demonstrated the persistence of agglutinating antibodies. It has however been impossible to determine whether the antibodies originated from the vaccination or from an enzootic infection. The serological response, as measured by a microtechnique of seroagglutination using a stained antigen, was studied in an isolated flock of 30 adult ewes. The trial period after the subcutaneous vaccination was 34 months, which included 3 lambings. Ten female offspring of these ewes were also studied. They were vaccinated by the conjunctival route and were studied for 18 months, which included 1 lambing. As is common for natural or experimental infection, high antibody titers were obtained 10–15 d after vaccination in both groups. The values then decreased and rose again to significant levels at each subsequent pregnancy. The vaccinal strain was never isolated from vaginal swabs taken at each lambing and there was no suggestion of stimulation by an external antigen. Gestation may therefore stimulate the antibody response. This suggests that the immunity conferred by this vaccination may last for at least 3 lambing periods. The consequences of these observations on vaccination protocols and serological diagnosis are discussed.

**vaccination / *Salmonella Abortusovis* / sheep / pregnancy**

**Résumé** — Vaccination par voie sous-cutanée ou conjonctivale avec une souche de *Salmonella Abortusovis* à virulence atténuée : effet de la gestation sur la réponse sérologique des brebis. Le contrôle annuel de troupeaux vaccinés par voie sous-cutanée avec une souche vivante à virulence atténuée de *Salmonella Abortusovis* a précédemment montré la persistance des anticorps agglutinants. Il était cependant impossible d'attribuer ces anticorps à la vaccination ou à l'infection enzootique. La réponse sérologique a été mesurée, par une micro-technique de séroagglutination

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avec un antigène coloré, pendant 34 mois incluant 3 périodes de gestation chez 30 brebis vaccinées par voie sous-cutanée, et pendant 18 mois incluant un agnelage chez 10 femelles issues de ces brebis et vaccinées par voie conjonctivale. Comme dans les infections naturelle ou expérimentale, des titres élevés ont été mesurés dès les 15 premiers jours avec les 2 voies. Ils déclinèrent ensuite pour atteindre de nouveau des niveaux significatifs à chacune des gestations suivantes. La souche vaccinale n'a jamais été isolée des écouvillons vaginaux prélevés à chaque agnelage, et aucune stimulation antigénique externe n'a été mise en évidence. Cette stimulation du système immunitaire à chaque gestation suppose que la protection conférée par ce vaccin pourrait persister sur au moins 3 agnelages. Les conséquences de ces observations sur les protocoles de vaccination et sur le diagnostic sérologique des maladies abortives sont discutées.

### **vaccination / *Salmonella Abortusovis* / Ovins / gestation**

## **INTRODUCTION**

*Salmonella enterica* subsp *enterica* ser Abortusovis (hereafter Sao), a sheep-adapted serotype, is one of the main causes of abortion in ewes and has been isolated in France and in several other countries (Pardon *et al*, 1988). Colonization of the foetoplacental unit with Sao leads to abortion with massive peripartum vaginal excretion, or sometimes a full-term lambing of infected live lambs.

A live attenuated vaccine using the strain Rv6 (Pardon *et al*, 1984) has been tested and has proved efficient in both experimental (Sanchis and Pardon, 1981; Pardon *et al*, 1990a) and natural conditions (Sanchis et Pardon, 1984b; Pardon *et al*, 1990b). A commercial form is now available (Salmovis ND, Rhône Mérieux, France).

Annual serological testing of vaccinated flocks in an enzootic area has demonstrated the persistence of agglutinating antibodies in nearly 50% ewes 3 years after subcutaneous vaccination with the commercial vaccine (Sanchis and Abadie, 1990). However under such conditions, it was difficult to determine whether these antibodies originated from vaccination or from enzootic infection.

To study the serological response to vaccination over a long period, we subcuta-

neously vaccinated 30 ewes in an isolated flock and followed the response for a period of 34 months, which included 3 lambings. Since conjunctival instillation of a virulent strain can induce an experimental abortive infection (Sanchis *et al*, 1991), we also followed the serological response after conjunctival vaccination of 10 ewes born from this flock for 18 months, which included 1 lambing. While the response declined for several months after vaccination in both groups, it became positively reactivated at each lambing in a high percentage of ewes.

## **MATERIALS AND METHODS**

### ***Animals and vaccinations***

Merinos x Préalpes ewes were reared in the specialized facilities at the laboratory (CNEVA, Sophia Antipolis). The flock was free of Sao infection and of the other main infectious causes of abortion (brucellosis, chlamydiosis, Q fever, border disease virus, *etc*). The ewes were mated with rams from the same flock, and lambings were synchronized.

- Thirty 3- to 4-year-old ewes were subcutaneously vaccinated 1 month before mating with 1 dose of the commercial vaccine ( $10^8$  viable Sao Rv6) according to the manufacturers' instructions (Rhône Mérieux, France).

Ten 8-month-old females born during the first lambing after vaccination of the adult ewes were vaccinated by the conjunctival route. They were mated 6 months later. On the basis of the dose required to induce an experimental infection by this route (Sanchis *et al*, 1991), each animal received  $10^9$  viable *Salmonella* Abortusovis strain Rv6 in two 50  $\mu$ l drops instilled between their right eye and the lower eyelid. For this vaccination, the vaccinal strain originating from the commercial preparation was cultured on trypticase soy agar (TSA: Diagnostic Pasteur, France) and suspended in phosphate-buffered saline (PBS, pH 7.2). Numeration on each vaccinal suspension before and after use did not show any significant difference with the expected number of viable *Salmonella*.

The vaccinated animals were reintroduced to their original flock and placed in contact with the older ewes, rams and lambs. They were kept outdoors in an enclosed yard during the day and observed each evening at housing.

### **Samplings and controls**

The blood of the subcutaneously vaccinated ewes was collected once a month beginning with the date of vaccination and extending over 34 months which included 3 successive lambings. Blood samples of the young ewes vaccinated by the conjunctival route were collected weekly during the first 2 months after vaccination, and monthly during the following 16 months, which included 1 lambing period.

Antibodies were quantified by the microagglutination test (MAT) using formalin-killed Sao ('H' antigen) stained with triphenyl-2,3,5-tetrazolium chloride (Prolabo, Paris, France). The microagglutination test and the techniques of cultivation and staining of the antigen have been described previously (Sanchis *et al*, 1985). The dilution required for positive activity was determined at the 1:320 with a 98.60% specificity (Sanchis and Abadie, 1990). Each series of reactions was standardized with a positive reference serum, and geometric means of titers with standard error were calculated for each group of animals. A comparison of means between the 8th and 14th months and between the 21th and 27th months following vaccination was done with the *t* test, and percentages were compared using a  $\chi^2$  test.

Four blood samples were collected each year from the whole flock for serological surveillance of the main causes of abortion and viral diseases (brucellosis, chlamydiosis, Q fever, toxoplasmosis, visna maedi virus, caprine arthritis encephalitis virus and border disease virus).

At each lambing, vaginal samples were collected by swabbing the vaccinated animals for bacteriological examination purposes including the detection of *Salmonella* Abortusovis, *Brucella*, *Listeria*, *Chlamydia* and *Coxiella*.

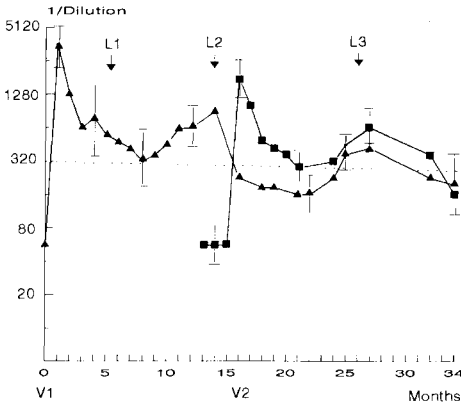
## **RESULTS**

The behaviour of the vaccinated animals was not affected by vaccination. A few subcutaneously vaccinated ewes showed a slight transient inflammatory reaction at the vaccinal injection site without abscess development. In the young ewes vaccinated by the conjunctival route, no eye or palpebral mucosa reactions were observed. There were no incidences of abortion and no specific pathogens were isolated during the entire duration of the experiment.

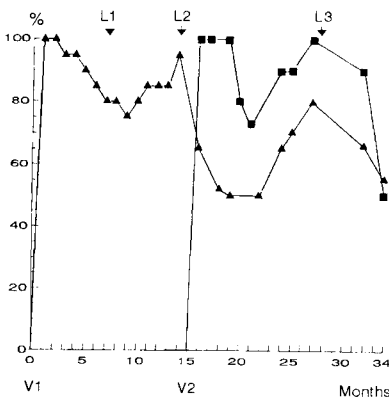
Before vaccination, the serological response measured by MAT was below 1/160 for all animals in the flock. One month after the subcutaneous vaccination, all ewes vaccinated by conjunctival route had titers between 1/1 280 and 1/10 240. Thereafter, serum titers decreased between each pregnancy, and rose again to significant levels at each lambing (fig 1). During the first lambing, 80% ewes remained positive with titers between 1/320 and 1/1 280 (fig 2). At the second lambing, 14 months after vaccination, titers of 90% ewes were between 1/1 280 and 1/5 120, and at the third 80% ewes had titers between 1/320 and 1/2 560. The differences between the lowest and highest means and percentages obtained during each phase of this evolution were highly significant ( $P < 0.02$ ).

The 10 young ewes vaccinated by the conjunctival route reached their highest antibody titers 10–15 d after vaccination. The

mean maximal titer was not significantly different from that reached in adult ewes after subcutaneous vaccination, although the vaccinal doses were different. The subsequent evolution was similar (fig 1): the decrease of titers during the next 6 months was followed



**Fig 1.** Kinetics of agglutinating antibodies after vaccination with the attenuated strain Rv6 of *Salmonella Abortusovis* (mean  $\pm$  SD). V1: vaccination of 30 adult ewes by the subcutaneous route ( $\blacktriangle$ ). V2: vaccination of 10 young ewes by the conjunctival route ( $\blacksquare$ ). L1, L2, L3: successive lambings.



**Fig 2.** Percentages of ewes that tested positively in the microagglutination test ( $> 1/320$ ) after vaccination with the attenuated strain Rv6 of *Salmonella Abortusovis*. V1: vaccination of 30 adult ewes by the subcutaneous route ( $\blacktriangle$ ). V2: vaccination of 10 young ewes by the conjunctival route ( $\blacksquare$ ). L1, L2, L3: Successive lambings.

by a new increase during their first pregnancy, and the titers of the 10 animals were between 1/320 and 1/2 560 at lambing. The titers then decreased and 50% animals had titers above 1/320 until the end of the observation (fig 2). The mean titers and percentages obtained at each phase of this evolution (months 21 and 27) were also significantly different ( $P < 0.01$ ).

The contact animals, which included aged ewes, rams and lambs, never produced antibodies against Sao (titers  $< 1/160$ ).

## DISCUSSION

The subcutaneous vaccinal dose of  $10^8$  viable bacteria was selected in accordance with previous studies (Pardon *et al*, 1990a) in order to avoid local inflammation. The dose of  $10^9$  viable bacteria used for vaccination by the conjunctival route was previously used for experimental abortion in ewes with the virulent strain (Sanchis *et al*, 1991) and has been shown to induce a regular serological response without adverse ocular reaction with the attenuated strain Rv6 (unpublished results).

In Sao infection, cellular immunity is involved in the mechanism of host resistance. However, circulating antibodies can also reduce the level of systemic infection in experimentally infected animals (Pardon *et al*, 1990a). Stimulation of the immune system can be recognized by the presence of antibodies. After subcutaneous vaccination, the serum titers increase dramatically and rapidly in a manner similar to the increase following an experimental infection with the virulent strain that uses the same routes (Pardon *et al*, 1983; Sanchis and Pardon, 1984a), or following abortion in natural infection (Sanchis and Abadie, 1990). Thereafter, the titers decrease progressively. However, as in an experimental infection outside the period of pregnancy (Sanchis and Par-

don, 1984a), the first lambing took place when more than 80% ewes still had high titers (fig 2). At each subsequent lambing a significant increase was again observed with titers remaining at the same level as those frequently observed in infected aged ewes in areas with enzootic salmonellosis (Sanchis and Pardon, 1984a).

The bacteriological examinations of swabs collected after lambings did not show any evidence of vaginal excretion of the vaccinal strain. These observations agree with those of previous results which indicate that the systemic dissemination of this strain is uncommon at this dose, and that viable vaccinal bacteria do not persist more than 1 month in the lymph nodes draining the site of injection (Pardon *et al*, 1990c; Fontaine *et al*, 1994). Moreover, in the course of this experiment none of the contact ewes, rams and lambs showed seroconversion suggesting an absence of cross-contamination with strain Rv6 during mating and lambing. Thus, the increase of antibody titers during pregnancies in vaccinated ewes seems to be independent of any excretion or external antigenic stimulation.

Such a serological evolution could be explained by 2 reasons which are not in themselves exclusive. First, the rise of antibody titer could be a result of a slight release of some bacteria from a chronic foci as a result of the altered state of immunoreactivity induced by pregnancy (Gill, 1985; Reynolds and Griffin, 1985). This release could lead to a reactivation of the immune system. The second explanation would be a general stimulation of the immune system, particularly the antibody response, as a result of the polyclonal response to different pathogens during pregnancy. This antibody evolution in relation to pregnancy has been observed in natural conditions (Russo and Malo, 1981; Sanchis and Abadie, 1990), and the rise of antibody titers at lambing may concern numerous pathogens. Thus, even in field conditions, an increase of

serum antibody titers at lambing without abortion is not necessarily a consequence of a new contamination during this period, but may be due to a restimulation of antibody production after a previous infection or vaccination.

The animals vaccinated by the conjunctival route were born from subcutaneously vaccinated ewes, but no agglutinating antibody was detected with the MAT before vaccination when they were 3–8 months old, showing that there was no cross-contamination from the vaccinated mothers to their offspring. Conjunctival vaccination involves the early colonization of the lymph nodes draining the mucosal site of translocation by the attenuated strain (MacCaughey *et al*, 1971; Plommet and Plommet, 1976). Besides a potential stimulation of the local immunity at the mucosal sites of translocation (MacCaughey *et al*, 1971; Tannock and Davis, 1973; Thomas and Harboume, 1974), this type of vaccination induces a serological response which is similar to that associated with the subcutaneous route (fig 1). Although the degree of protection following conjunctival vaccination with Sao has not yet been assessed, it is probable that, as described for brucellosis (Plommet and Plommet, 1976; Fensterbank *et al*, 1982), the serological response induces a similar level of protection as does the subcutaneous route.

The results of the present report provide information about the duration of immunity following vaccination with strain Rv6. After an initial vaccination of the whole flock, if the increase of circulating antibody observed during the next lambing indicated a sufficient level of immunity, it might be possible to limit further vaccination to new young ewes kept or introduced into the flock. The evolution of antibody titers reported in this study confirms the importance of sampling sera in the first 2 months following lambing for diagnostic purposes. Despite a possible lower dose of vaccine by conjunctival route

leading to lower serum titers (Plommet and Plommet, 1976), the results also emphasize the necessity of using quantitative serological tests to distinguish abortion or recent infection from vaccination or previous natural infection. Furthermore, in flocks subjected to various abortive pathogens, the probable polyclonal stimulation of the immune system at lambing implies the necessity to perform differential serological analyses of the main abortion causes in order to discriminate the cause of a given abortion from other previous enzootic abortive infections.

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