

RESPONSE OF EWES TO TEMPERATURE-SENSITIVE MUTANTS OF *CHLAMYDIA PSITTACI* (VAR *OVIS*) OBTAINED BY NTG MUTAGENESIS

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Résumé

CONSÉQUENCES POUR LA BREBIS DE L'INOCULATION DE CHLAMYDIA THERMOSENSIBLES OBTENUES PAR MUTAGENÈSE A LA NITROSOGUANIDINE. — Deux souches thermosensibles 1B et 1H de *Chlamydia psittaci* d'origine ovine, obtenues par mutagenèse, ont été utilisées comme vaccin vivant. Pour cela 20 agnelles ont été vaccinées avec $3,9 \times 10^6$ UFP de 1B et 20 agnelles avec $3,5 \times 10^6$ UFP de 1H, onze semaines avant la saillie. Les conséquences de la vaccination ont été étudiées pendant la gestation suivante au moyen du titre en anticorps fixant le complément, de l'excrétion des chlamydia et des résultats de la mise-bas. La vaccination n'a pas perturbé la gestation et aucune brebis n'a excrété de chlamydia. L'immunité des brebis a été éprouvée, un an après la vaccination, par inoculation de $2,5 \times 10^6$ UFP de chlamydia virulentes (souche parentale) à 78 jours de gestation. Alors que 9/10 des brebis non-vaccinées ont excrété des chlamydia à la mise-bas, et 4/10 ont avorté, seulement une des 18 brebis vaccinées avec 1H et aucune des 16 brebis vaccinées avec 1B ont excrété des chlamydia à la mise-bas. La souche 1B pourrait être utilisée comme vaccin vivant après d'autres contrôles d'inocuité et d'efficacité.

Chlamydiosis is one of the main causes of ovine abortion in France. Although it was found that the immunity following a primo-infection is strong enough to prevent excretion of chlamydia after challenge (Rodolakis and Souriau, 1980) vaccination with inactivated chlamydiae emulsified in adjuvant is often disappointing (Philipp *et al.*, 1978 ; Linklater and Dyson, 1979 ; Rodolakis and Souriau, 1979). An avirulent live-vaccine administered before breeding could be

the key to the control of chlamydial abortion in ewes.

As the virulence of chlamydiae attenuated by serial passages on cell cultures or in the yolk sac of chicken embryos was generally restored from the first passage in animals (Becerra *et al.*, 1976) and as temperature-sensitive viruses have been widely used as vaccine (Richman and Murphy, 1979) we tried to isolate temperature-sensitive mutants. Two *ts* strains were

isolated after treatment of virulent abortive ovine strain *Chlamydia psittaci* AB7 with N-methyl-N'-nitro-N-nitrosoguanidine (NTG). On McCoy cells, these two *ts* strains had the same optimum growth temperature, 38 °C, as the parental strain. At the restrictive temperature, 39.5 °C *ts* strains differed from the parental strain in their total infective yield of chlamydiae, their plating efficiency and the morphology of the plaques and cytoplasmic inclusions. Their viability at 51 °C was also shorter than those of wild field strains. Their virulence for pregnant or non-pregnant mice was reduced and when inoculated in non-pregnant mice they induced a strong immunity against parental or abortive field strains (Rodolakis, 1982).

To study the possibility of using these *ts* strains as live-vaccine, ewes were vaccinated 11 weeks before service. The consequences of the vaccination were studied during the subsequent pregnancy, and immunity was challenged one year after vaccination during the second gestation of the ewes.

Materials and Methods

Chlamydial strains

The AB7 strain of *Chlamydia psittaci* (var *ovis*) was used as wild type chlamydia. It was originally obtained from Dr. P. Faye in 1972 (Laboratoire de Pathologie du Bétail, Ecole Nationale Vétérinaire, Maison-Alfort, France), then maintained as a reference strain in this laboratory. Its second passage in chicken embryo, after isolation from an aborted lamb, was used as inoculum.

The temperature-sensitive mutants (1B and 1H) were obtained by treatment with 10 µg/ml of Nitrosoguanidine 24 h after the inoculation of the McCoy's monolayers with the AB7 strain of *Chlamydia psittaci*. They were plaque-purified three times. Their plating efficiency (35 °C/39.5 °C) was respectively 1×10^1 and 1×10^3 (Rodolakis, 1982). They were selected at 35 °C as permissive temperature, but their optimum growth temperature on McCoy cells was 38 °C as for the wild type AB7 strain.

After being plaque-purified three times on McCoy cells, their first passage in chicken embryo was used as inoculum.

Experimental ewes

Fifty-seven yearling « Berrichone » ewes were selected from the Station de Pathologie de la Reproduction, flock which had no history

of enzootic abortion. However, a large sample of ewes taken at random before the initiation of the experiments gave low titers in the complement fixation test with chlamydial antigen (1/10-1/40).

Vaccination

Twenty of the 57 ewes taken at random received 2 ml containing 3.5×10^6 PFU of 1H strain (Banks *et al.*, 1970). This inoculum was injected intradermally at five to seven sites on the thoracic skin behind the front legs, 11 weeks before service. Twenty ewes received 3.9×10^6 PFU of 1B strain, in the same conditions, and 17 ewes were kept as controls and bred at the same time.

After vaccination, ewes were kept in separate pens in a disease security building.

Challenge

Thirteen months after vaccination all the ewes were mated. The resistance of the ewes was challenged by intradermic inoculation with 2.5×10^6 PFU of AB7 strain in the opposite side to where the vaccination was performed at 78 days of pregnancy.

Clinical examination

Animals were observed daily for clinical signs of disease throughout the duration of the experiment. Their rectal temperature was recorded one day before and six days following vaccination and nine days following challenge.

Bacteriological determination

Three days following lambing, swabs from ewes were used for isolation assays in cell culture by plaque assay on McCoy cells (Rodolakis and Chancerelle, 1977).

Serological tests

Group-specific chlamydial complement fixing (CF) antibody titers were determined by the microtiter technique in the sera of ewes (Rodolakis *et al.*, 1977) with a yolk sac-propagated chlamydial antigen (Rakeia, Roger Bellon, France). The highest serum dilution showing less than 50 % hemolysis was taken as the end-point. A serum was considered positive when its end-point was 1/80 or greater.

Blood samples for serum collection were taken before vaccination at seven day intervals four weeks following vaccination and subsequently at fourteen day intervals.

Results

Response to vaccination

During the 24 h after vaccination the ewes had a mild-rise in temperature : 0.5 in average. Only one ewe vaccinated with 1H strain had a biphasic temperature response identical with those observed after inoculation with virulent parental strain. We must note that at the time of vaccination, the mean temperature of the ewes

was high, 40 °C, and remained at this level during the whole week.

During this week all the ewes had a temperature of at least 39.5 °C.

The vaccination did not affect the fertility of ewes (table 1). The duration of pregnancy was the same in the vaccinated and control groups, and there was also no difference between the mean total weight of lambs per ewes (table 1).

Table 1. — Consequences of vaccination on the subsequent gestation of ewes (first gestation)

	Groups		
	Control	Vaccinated with 1B 3.9×10^6 PFU	Vaccinated with 1H 3.5×10^6 PFU
Number of ewes			
vaccinated	17	20	20
pregnant	14	18	17
Lambs			
live	17	16	16
dead	5	7	5
age (days) (mean \pm s.e.)	145.7 ± 2.8	145.8 ± 1.4	145.6 ± 2.1
weight (kg)	4.8 ± 1.5	4.7 ± 1.2	4.8 ± 1.5

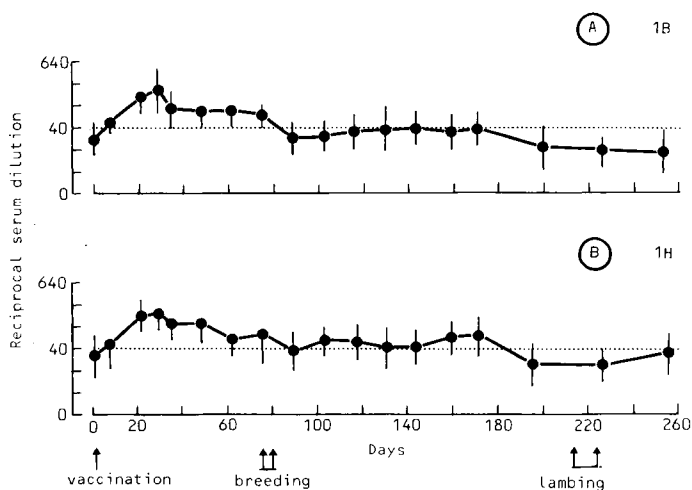


Fig. 1. — Complement fixing antibody response of ewes to vaccination with temperature-sensitive chlamydia.

A : 1B strain 3.9×10^6 PFU per ewe.

B : 1H strain 3.5×10^6 PFU per ewe.

In the three groups, those vaccinated with 1B and 1H strains, and the control 7/23, 5/21 and 5/23 lambs respectively were stillborn or died within three days following delivery.

Chlamydiae were not isolated from these lambs or from vaginal swabs taken within three days following lambing. These dead lambs could be considered as the normal lost during lambing of Berrichones ewes.

Antibody titers of the ewes varied from 1:80 to 1:320 during the three weeks post-vaccination and a gradual decline occurred thereafter until they became negative again ($\leq 1:40$) in about six months. There was no increase in CF antibody titers after lambing (fig. 1).

Response to challenge inoculation

The ewes became febrile within 24 h after challenge (fig. 2). There was no difference in magnitude and duration of the temperature response during the first 48 h, but an important difference in pattern of the mean temperature response between vaccinated and control ewes was noticed the fourth day: vaccinated ewes had a monophasic temperature response whereas it was biphasic in the case of the control ewes.

In the control group the inoculation induced abortion in 4/10 ewes and the excretion of chlamydia at lambing in 9/10 ewes. The ewe which did not excrete chlamydia had a CF antibody titer of 1:320 four weeks after inoculation. A gradual decline in this titer occurred thereafter. At lambing, its titer was 1:80 and it gave birth to a live lamb after 143 days of gestation.

All the control ewes responded to the challenge inoculation by an increase in CF antibody titer ranging from 1:160 to 1:640 (fig. 3). Eight out of ten control ewes had a CF antibody titer ranging from 1:80 to 1:320 at lambing or during the three weeks following lambing. The two others which gave birth to live lambs but excrete chlamydia had a titer of 1:20 and 1:40 during the month after they lambled.

The duration of pregnancy was significantly shorter in control groups (140.9 days) than in vaccinated groups (143.8 and 143.6 days) (table 2).

In the group vaccinated with 1B strain, none of the 16 ewes excreted chlamydiae at lambing, or had an abnormally short pregnancy. Four of them produced stillborn or weak lambs which died within 72 h: two of them had a hard lambing which involved the death of lambs, the

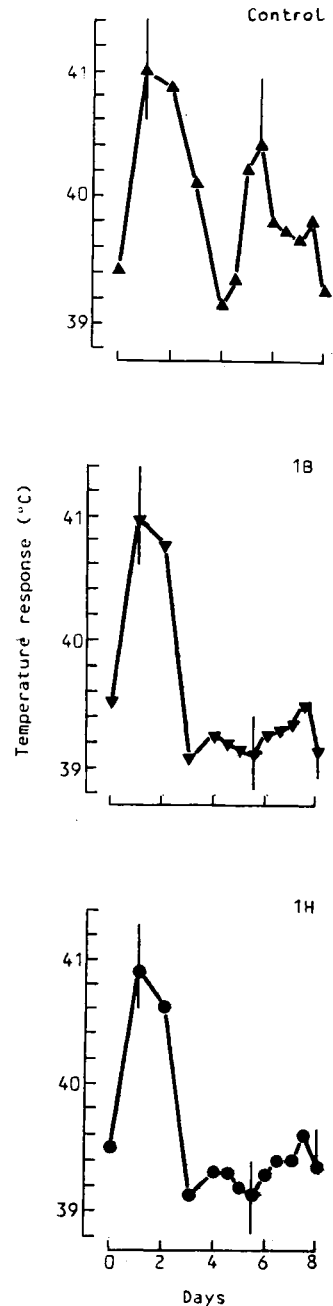


Fig. 2. — Temperature response of ewes following challenge with virulent *Chlamydia psittaci*: vaccinated ewes with 1H strain (●) or 1B strain (▼) and control ewes (▲).

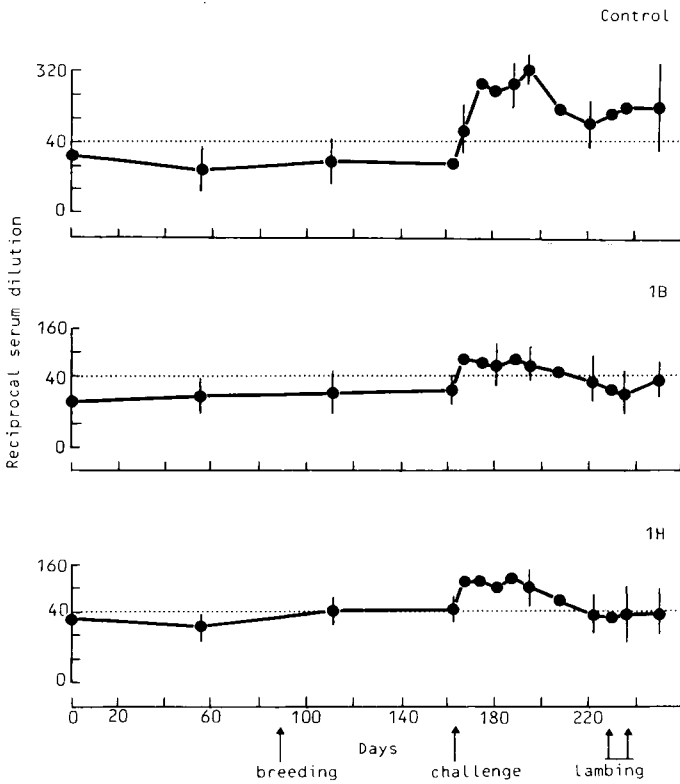


Fig. 3. — Complement fixing antibody response of ewe to challenge with virulent chlamydiae. Control ewes : or ewes vaccinated with 1B strain or with 1H strain.

Table 2. — Response of ewes to intradermic challenge with 2.5×10^6 PFU of *Chlamydia psittaci* AB7 at 78 days of pregnancy (second gestation)

	Groups		
	Control	Vaccinated with 1B 3.9×10^6 PFU	Vaccinated with 1H 3.5×10^6 PFU
<i>Number of ewes</i>			
pregnant	10	16	18
excreting	9	0	1
<i>Lambs</i>			
live	7	23	17
dead	11	8	12
age (days) (mean \pm s.e.)	140.9 \pm 6.1	143.8 \pm 1.2	143.6 \pm 3

third produced four lambs which did not survive and the fourth produced normally two lambs one of which died 24 h later without any apparent reason. None of the ewes had a rise in CF antibody titer at lambing.

In the group vaccinated with 1H strain, 1/18 ewes excreted chlamydiae after giving birth to a live lamb at 143 days of gestation. This ewe was the only one of the group which had a rise in CF antibody titer at lambing. Two ewes had an abnormally short gestation; one had four dead lambs after 137 days of gestation, and the other had two dead lambs at 139 days of pregnancy. Neither of them excreted chlamydia at lambing. Three other ewes produced stillborn lambs but no chlamydiae were isolated from these three ewes, one of which had a stillborn lamb weighing 1.8 kg.

Discussion

We infected ewes prior to breeding with *ts* strains of *Chlamydia psittaci*. The immunity of these ewes was challenged by intradermic inoculation at two months of gestation one year after vaccination. The two *ts* strains did not disturb the subsequent gestation when they were inoculated in ewes 11 weeks before mating. This vaccination induced good immunity since none of the 16 ewes vaccinated with 1B and only one out of the 18 vaccinated with 1H excreted chlamydiae after challenge, although in the same conditions nine out of ten control ewes excreted chlamydiae.

After vaccination the ewes remained clinically normal and their rise in temperature was very low compared to those observed with AB7 strain (Bernard, 1976). The *ts* strains failed to infect the placenta and the foetus when the ewes were vaccinated before mating and there was no chlamydia excreted at lambing. The CF

antibody titer of vaccinated ewes became negative again six months after vaccination.

In contrast to the ewes vaccinated with live chlamydiae adapted to chicken embryos or tissue culture (Becerra *et al.*, 1976), the vaccination with 1B strain induced protection in pregnant ewes as strong as is induced by a primo-infection (Rodolakis and Souriau, 1980) and really different to the protection observed after vaccination with a killed commercial vaccine (Rodolakis and Souriau, 1979). Since none of the 16 vaccinated ewes excreted chlamydiae, the challenge was done with parental strains and one could argue that the protection may be limited to parental strains, but our results on mice showed that 1B strain protected against challenge of bovine or caprine origine. At present, its safety and efficacy against a challenge of caprine origine is being studied in goats.

These results seem to direct our choice towards 1B strain as a potential live vaccine since 1H strain was less efficacious and less stable, as indicated by our results on mice. But the 1B strain needs further investigations on its safety and efficacy.

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Summary

Two temperature-sensitive strains, 1B and 1H, of ovine *Chlamydia psittaci* obtained by mutagenesis were used as live vaccine: eleven weeks before breeding, 20 ewes received 3.9×10^6 PFU of 1B strain, and 20 others 3.5×10^6 PFU of 1H strain. The consequences of the vaccination were studied during pregnancy by recording CF antibody titer, chlamydial vaginal excretion and lambing performance. The vaccination did not disturb pregnancy and none of the ewes excreted chlamydiae at lambing. The immunity of the ewes was challenged one year after vaccination, by intradermic inoculation of 2.5×10^6 PFU of the virulent parental strain at 78 days of pregnancy, while nine out of ten control ewes excreted chlamydiae at lambing, none of the 16 pregnant ewes vaccinated with 1B strain, and only one the 18 pregnant ewes vaccinated with 1H strain did so. After further controls of safety and efficacy, 1B strain could provide a live vaccine against abortive chlamydiosis.

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