CLINICAL EVALUATION OF A COMMERCIAL VACCINE AGAINST CHLAMYDIAL ABORTION OF EWES

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Résumé

MESURE DE L'EFFICACITE D'UN VACCIN CONTRE LA CHLAMYDIOSE ABORTIVE OVINE : ETUDE CLINIQUE. — L'immunité de brebis vaccinées avant l'insémination avec un vaccin adjuvé préparé à partir de chlamydia cultivées sur œuf embryonné, est éprouvée à 70 jours de gestation par inoculation, par voie intraveineuse (IV) ou intradermique (ID), de 6×10^7 chlamydia virulentes cultivées sur œuf. Les conséquences de l'inoculation d'épreuve sont suivies par la température rectale, le titre en anticorps fixant le complément, le résultat des mise-bas et la recherche des chlamydia dans les écouvillons vaginaux. Le vaccin ne protège pas contre une épreuve virulente administrée par voie IV : on note 7 avortements parmi les 10 brebis du lot vacciné, et 7 parmi les 8 brebis témoins. Lorsque l'épreuve virulente est administrée par voie ID, l'incidence des avortements est réduite : 4 avortements parmi les 9 brebis vaccinées, et 9 parmi les 9 brebis témoins. Cependant, des chlamydia sont isolées dans les écouvillons vaginaux de 7 de ces 9 brebis vaccinées.

Chlamydial abortion is the main cause of reproductive failures in ewes in France (Fontaine, 1975). Chlamydiae are susceptible to several antibiotics, tetracycline, rifamicin, chloramphenicol, and the incidence of abortion can be reduced by these chemotherapeutic agents ; but they have to be present at a high concentration in the blood in order to be effective in preventing chlamydial abortion (Mitscherlich and Liess, 1957 ; Frank *et al.,* 1962 ; Storz, 1971). This makes it difficult to use chemotherapy alone in chlamydial prophylaxis. Vaccination, if efficient, would be very valuable. Vaccines against chlamydial abortion are available in France. However the lambing performance observed in flocks after vaccination is inconsistent. In order to explain these results we studied the lambing performance and the shedding of chlamydiae in vaginal mucus of vaccinated or control ewes. These animals had received virulent chlamydiae by the intravenous (IV) or the intradermal (ID) route during pregnancy. We found that vaccine did not protect against IV challenge. Yet

when the challenge was given by the ID route the incidence of abortion was reduced but not the shedding of chlamydiae.

Materials and methods

Experimental design

Prealpes x Lacaune 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 to the complement fixation test with chlamydial antigen (1/10 -1/40).

First experiment

Forty ewes divided in two groups, received either 2 ml of a commercial vaccine (adjuvant vaccine prepared from yolk sac propagated chlamydiae, Rikevac Roger Bellon) subcutaneously (SC), two weeks before service, or were kept as control and bred at the same time. Eighteen days after breeding five vaccinated and four control ewes were diagnosed as pregnant and were challenged by the IV route at 70 days of gestation. The others were reinseminated five weeks after the first insemination and the pregnant ewes (5 vaccinated and 4 controls) were challenged by the IV route 70 days later. The first diagnosis of pregnancy was incorrect, so that some of the ewes (as indicated in table 1) which were pregnant at the first service, were in fact inseminated twice. These ewes were rechallenged at 105 days of pregnancy instead of 70, made with exactly the same inoculum.

After breeding, infected ewes were kept in separate pens in a Disease Security Building.

Second experiment

Forty ewes divided in two groups received either 2 ml of vaccine Rikevac by the SC route five weeks before service, or were kept as control. The ewes diagnosed as pregnant (nine vaccinated, nine control) were challenged by the intradermic route (ID) at sixty-eight days of gestation.

These ewes were housed together in a Disease Security Building.

Challenge

A yolk sac propagated chlamydial strain AB7 (Faye *et al.,* 1971) in its 2nd passage in chicken embryo after isolation from an aborted lamb was used as inoculum. It contained 3×10^7 plaque forming units (PFU)/ml (Banks *et al.,* 1970).

In the first experiment the ewes received 2 ml of the inoculum by the jugular route. In the second experiment, the inoculum (2 ml) was injected intradermally at five to seven sites on the thoracic skin behind the front leg, opposite the side where the vaccination was done.

Clinical examination

Animals were observed daily for clinical signs of disease throughout the duration of the experiment and their rectal temperature was recorded for three days before and nine days following challenge.

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 per cent hemolysis was taken as the end point. A serum was considered positive when its endpoint was 1/80 or greater, and negative when the endpoint was 1/20 or lower.

Blood samples for serum were taken before vaccination and at fourteen day intervals following, then each week following the challenge.

Bacteriological determination

Impression smears were prepared from representative cotyledons and fluids or organs from fetuses and examined for the presence of chlamydiae after staining by the Stamp method.

Swabbings from ewes and fluids (exudates) or organs from fetuses were used for isolation assays in cell culture. In the first experiment, it was done by demonstrating cytoplasmic inclusions in stained Hela 229 cells ; in the second, by plaque assay on Mc Coy cells (Rodolakis and Chancerelle, 1977).

Results

Clinical Response

All animals become febrile within 24 hr after inoculation with essentially no difference in

temperature between vaccinated and control subjects. Among the 36 inoculated ewes, 26 had a biphasic temperature response (fig. 1, table 1 and 2). This curve was characterized by a second peak on the third or fourth day after IV inoculation, and by an additional peak on the sixth day after ID inoculation.

There was no difference in the pattern of the mean temperature response between vaccinated and control ewes challenged by the IV route. However, an important difference was noticed after the ID challenge (fig. 1) : in that the mean temperature of the vaccinated ewes was monophasic instead of biphasic as it was in the control ewes. Another difference between IV and ID inoculation was that the duration of the first peak of fever was, on the average, 30 hr after IV inoculation and 72 hr after ID inoculation.

For 5 to 6 days post inoculation the ewes became depressed and inappetent. Two of them (6016 and 6020) were observed to be lame from 72 hr after challenge until the end of the experiment. Otherwise the ewes recovered rapidly and remained clinically normal until the onset of abortion. Specific clinical signs could not be observed in infected ewes until abortion was imminent, except for some ewes which discharged a thick, reddish brown material from the vagina several days before abortion.

After inoculation with virulent chlamydiae by the IV route, 7 of 8 control and 7 of 10 vaccinated ewes aborted (table 1). The observed difference between the 2 groups was not significant (Mainland *et al.*, 1956). If we take only the ewes inoculated at 70 days of gestation into account, we notice that 6 of 6 controls and 4 of 6 vaccinated ewes aborted. This difference was not significant by the exact probability test.

In the second experiment, all 9 control ewes aborted after ID challenge, whereas in the vaccinated group 5 ewes lambed normally, 3 aborted, and one gave birth to a weak lamb (table 2). The latter was classed as an abortion. The observed difference between the lambing performance of vaccinated and non vaccinated

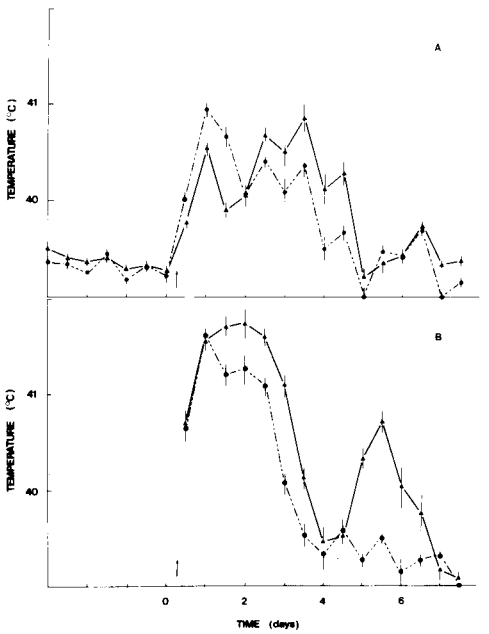

Fig. 1. - Temperature response of ewes following intravenous (Fig. 1 A) or intradermal (Fig. 1 B) challenge with 6×10^7 chlamydiae.
vaccinated ewes ● - - ● ; control ewes ▲ — ▲ ; arrow : day of inoculation.

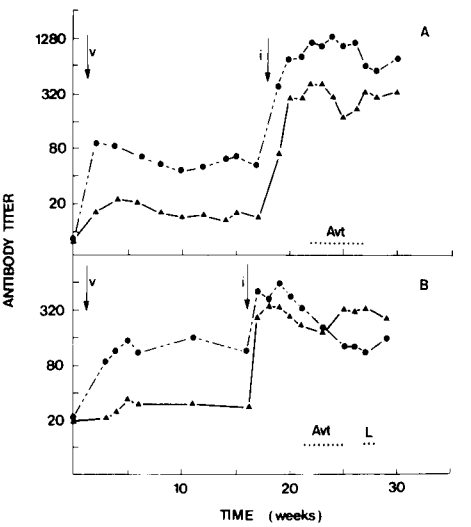

Fig. 2. - Complement fixing antibody response of ewes following intravenous (Fig. 2 A) or intradermal (Fig. 2 B) challenge with 6×10^7 chlamydia.
vaccinated ewes ● - - ● ; control ewes ▲ — ▲ ; V : vaccination ; I : challenge ; L : lambing time ; Avt : abortion time.

Table 1. Response of ewes inoculated by the intravenous route

Ewe	Temperature Response			Antibody [a] Response Postvaccination	Foetus		Bacteriological Results		
	Biphasic	Maximum °C			Age (days)	Condition [b]	Stamp Staining (placenta)	Isolation [c]	
		1 st Peak	2 nd Peak					Swab	Other
Group : *control*									
3014	+	40.2	40.9		138	D	+	nt	G^+ Pe^+
5043	+	40.1	41.5		135	D	nt	nt	nt
5056	+	40.5	40.4		142	D	+ + +	nt	G^+
7213	+	40.5	41.4		124	DD	nt	nt	nt
719[d]	+	40.7	41.2		110	DDD	nt	nt	G^+ Pe^+
4017[d]	+	41	41.4		138	D	nt	nt	G^+
3064[e]	–	40.7	-		144	LL	+ +	nt	nt
7249[e]	+	40.9	41.9		137	WW	+	+ + +	nt
Group : *vaccinated*									
3113	+	40.5	41.3	160	125	DD	nt	nt	nt
5039	+	40.7	40.4	80	141	L	+ + +	+ +	nt
5044	–	41.6	-	80	145	L	nt	+	nt
7208	+	41.3	40.6	160	81	D	nt	+ + +	G^+
7244	+	40.6	41.7	80	125	D	nt	nt	nt
3044[d]	+	41.6	41	320	113	D	nt	nt	Pe^+ G^+
4030[e]	+	41.5	40.9	80	137	D	+	+ + +	nt
7242[e]	+	40.7	40.6	80	137	W	nt	nt	nt
7271[e]	–	41.1	-	80	139	DL	+ +	+ + +	G^+
7293[e]	–	41.1	-	160	143	L	+ + + +	+ + +	nt

a) titers expressed as the reciprocal of the highest serum dilution giving 50% hemolysis in CF test ; b) condition of foetus D : dead, W : weak, L : live ; c) nt : not tested, cont. : bacterial contamination, G : gastric fluid, Pe : peritoneal fluid, Li : liver ; d) ewes inoculated at 70 days of gestation at the same time
as those inoculated at 105 days of gestation ; e) ewes inoculated at 105 days of gestation ; f) the foetus of ewe n° 6010 was not found but this ewe had an abundant reddish-brown vaginal discharge at 130 days of pregnancy.

Table 2. Response of ewes inoculated by intradermal route

Ewe	Temperature Response			Antibody [a] Response Postvaccination	Foetus		Bacteriological Results		
	Biphasic	Maximum °C			Age (days)	Condition [b]	Stamp Staining (placenta)	Isolation [c]	
		1 st Peak	2 nd Peak					Swab	Other
Group : *control*									
6003	+	42	41.5		137	DD	+ + +	nt	G-
6007	+	41.6	41.1		126	DD	+ + +	+ + +	-
6009	+	41.7	41.2		76	D	±	cont	nt
6012	+	42.1	41.2		77	D	+ + +	-	nt
6014	+	42.2	40.7		135	D	-	+	nt
6015	+	42	40.8		134	D	-	+	G-
6016	–	41.4	-		134	DD	nt	+ + +	G-
6020	+	42.2	41		80	DD	+	-	Li^+
6010[f]	+	41.9	41.3		?	?	nt	nt	nt
Group : *vaccinated*									
6002	+	42.2	40.9	160	75	DD	+ +	cont	nt
6005	+	41.5	40.2	80	132	DD	+ +	cont	+
6006	–	42.1	-	80	142	LL	nt	-	nt
6008	+	41.4	41.6	160	141	LL	nt	+ +	nt
6021	–	41.3	-	80	144	W	nt	-	G cont
6028	–	41.8	-	160	144	L	nt	+	nt
6030	–	42	-	160	142	LL	nt	-	nt
4019	–	41.4	-	160	144	L	nt	+ + + +	nt
5026	+	41.5	40.9	320	126	DD	+ + +	+	G-

a) b) c) f) see table 1.

ewes was significant ($P < 0.05$) by the exact probability test.

The magnitude of the temperature response had no effect upon the condition of lambs at birth (table 1 and 2). However a highly significant ($P < 0.002$) difference in incidence of live lambs was observed between ewes with a monophasic temperature response and ewes with a biphasic temperature response (table 3).

Antibody response

Antibody titers of the ewes varied from 1 : 80 to 1 : 320 by 2 weeks post vaccination and remained positive until challenge.

After IV inoculation, the mean antibody titers of vaccinated ewes were, on the whole, higher than those of the control ewes. The antibody titers were maximum at 4 to 5 weeks after challenge. A gradual decline in the titers occured thereafter until the onset of abortion, when a second rise in antibody was recorded (fig. 2A).

The ID challenge induced a rise of antibody titers in the vaccinated and non vaccinated ewes (fig. 2B). The mean titer attained its maximum 3 weeks post inoculation in both cases. It declined gradually thereafter in all the vaccinated ewes, but it increased again after abortion in the control.

There was no correlation between the magnitude of the antibody response to the vaccination and to the challenge, and the pattern of the thermic response or the lambing performance of the ewes (table 1 and 2).

Bacteriological results

Chlamydiae were shed profusely in fetal placenta and vaginal fluids (table 1 and 2), while vaginal swabbings of 6 of the 8 vaccinated ewes which lambed normally were positive for chlamydiae. the chlamydial excretion continued for at least 8 days in two of them (7242 and 6008). Only two vaccinated ewes, 6006 and 6030, were found negative on both bacterioscopic and bacteriologic tests.

Table 3. - Relation between lambing performance and febrile response of ewes.

Temperature Response	Lambing performance		Total
	Normal	Abortion	
Biphasic	2	24	26
Monophasic	7	3	10
Total	9	27	36

$\chi^2_c = 11.81$

Discussion

The immunity of ewes vaccinated with an adjuvant vaccine preparated from yolk sac propagated chlamydiae prior to breeding was challenged by either intravenous or intradermal inoculation at 70 days of pregnancy. The events following challenge were traced by recording rectal temperature, CF antibody titer, lambing performance and chlamydial isolation from vaginal swabs.

The intensity and the duration of the febrile response of ewes to challenge were in agreement with published data (Storz, 1963 ; Pierce *et al.*, 1963 ; Studdert and McKercher, 1968 ; Novilla and Jensen, 1970 ; Becerra *et al.*, 1976). However contrary to the findings of these authors, we found that a biphasic response was the general rule and we think that this pattern is releated to the pathogenic events leading to placental and fetal chlamydial infection. When pregnant ewes were inoculated by the intravenous route, after an initial clearance the rate of which depended on the amount of the inoculum, chlamydiae could not be recovered from the blood and organs for a period of at least eighteen hours (Storz *et al.*, 1968). Then chlamydiae appeared in different organs. The chlamydial multiplication in these organs gave rise to a secondary blood infection phase during the ensuing seventy two hours. This phase of generalized systemic infection is transient according to Storz *et al.*, (1968). We thought it could induce the 2nd febril peak. After intradermal inoculation, the chlamydiae should multiply in the local lymph node(s) leading to a blood infectious phase which would allow the chlamydiae to reach the placenta and fetus. This chlamydemia could promote the 2nd temperature peak which would be equivalent to the initial one in IV inoculated ewes. When the ewes were vaccinated prior breeding and challenged by the ID route, the immunity could prevent the chlamydemia and the advent of the 2ndary febrile peak.

The difference in response of vaccinated

ewes between the 1st and 2nd experiment could be ascribed to the route of challenge. The IV route by-passed the lymph nodes when multiplication and/or inhibition determine the evolution of the disease : the sensitized lymphocyte produces various lymphokines among which are those which promote and enhance phagocytic activity. Thus the chlamydiae deposited in the skin are disposed of by phagocytosis before they can reach the general circulation and invade the placenta and the fetus. These data are in agreement with those of McKercher *et al.,* 1973) on the experimentally induced immunity to chlamydial abortion in cows which showed that vaccination can protect against ID but not against IV challenge.

Storz (1963, 1971) and Shukla (1969) showed that no relationship between complement-fixing antibodies and immunity to chlamydial infection could be found. Our results confirm these data : two ewes responding to vaccination with a maximum titer of 1 : 320 (ewes n° 5026 - 3044) aborted, whereas two other ewes responding to the vaccination with the minimum titer of 1 : 80 (ewes n° 6006 - 5039) lambed normally. Thus, the CF test could not be used to evaluate the protective effect of the vaccine. We have not yet tested for the presence of neutralizing antibodies, which are not related to CF antibodies (Mc Ewen and Foggie, 1954 ; Shukla, 1969 ; Storz, 1971) and may be more significant as an indication of immunity.

In the 2nd experiment, we found that vaccination of the ewes prior to breeding reduced the incidence of abortion to intradermal challenge but could not prevent the excretion of chlamydiae. Therefore vaccination may delay, but not prevent, colonization of the placenta. It should be observed for example, that vaccinated ewes n° 4019, after delivering a live lamb, had a vaginal discharge which contained as much chlamydiae as the challenge inoculum (ie 10^7 PFU). This may explain the failure of some vaccination programs on a flock basis.

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Summary

The immunity of ewes vaccinated prior to breeding with an adjuvant vaccine prepared from yolk sac propagated chlamydiae, was challenged at 70 days of pregnancy by intravenous (IV) or intradermal (ID) inoculation of a suspension of virulent yolk sac propagated chlamydiae. The response to challenge inoculation was followed by recording rectal temperature, CF antibody titer, lambing performance and by chlamydial isolation from vaginal swabs. That vaccine did not protect against IV challenge : 7 of 10 vaccinated ewes, and 7 of 8 controls aborted. When the challenge was given by the ID route, the incidence of abortion was reduced : 4 of 9 vaccinated, and 9 of 9 control ewes aborted, but chlamydiae were isolated from vaginal swabs of 7 of the 9 vaccinated ewes.

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