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COMPARISON BETWEEN SUBCUTANEOUS AND CONJUNCTIVAL ROUTE OF VACCINATION WITH REV. 1 STRAIN AGAINST *BRUCELLA MELITENSIS* INFECTION IN EWES

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Résumé

COMPARAISON ENTRE VACCINATION PAR VOIE SOUS-CUTANÉE ET CONJONCTIVALE AVEC LA SOUCHE REV.1 CONTRE L'INFECTION A *BRUCELLA MELITENSIS* DES BREBIS. — Dans les zones d'élevage ovin où la prévalence de l'infection à *Brucella melitensis* est élevée, l'assainissement ne peut être acquis sans le secours de la vaccination. L'utilisation de la souche vaccinale *Brucella melitensis* Rev.1 confère une très bonne immunité. L'inconvénient est l'induction d'anticorps persistant longtemps et pouvant perturber le diagnostic sérologique au moment où, après plusieurs années de vaccination, il est nécessaire de revenir à la prophylaxie sanitaire. Il est donc important de rechercher un autre mode de vaccination ne présentant pas cet inconvénient.

Dans cette expérience, l'efficacité de la vaccination par voie conjonctivale en une inoculation de $1,4 \times 10^8$ Rev.1 suivie d'un rappel de $2,9 \times 10^8$ Rev.1 six mois plus tard, a été comparée à celle de la vaccination normale par voie sous-cutanée avec $1,4 \times 10^9$ ou $2,7 \times 10^9$ Rev.1.

La vaccination par voie conjonctivale a conféré une protection très légèrement supérieure à celle donnée par la vaccination normale, et avec une réponse sérologique plus courte. Il est possible qu'une vaccination unique par voie conjonctivale, évitant une double manipulation des animaux et donnant peu d'anticorps, soit suffisante pour assurer une bonne protection. Les recherches devraient être poursuivies dans ce sens.

Most workers agree that ovine brucellosis cannot be eradicated in heavily infected areas by test and slaughter policy alone and that vaccination is the most practical method to control the disease. *Brucella melitensis* strain Rev.1 vaccine

is one of the best vaccines available. It affords an excellent immunity (Alton and Elberg, 1967; Elberg, 1981) but induces long-lasting antibody titers of diagnostic significance. This acts as a drawback which is difficult to reconcile with the

inescapable stage of test and slaughter policy following a program of vaccination lasting several years.

A « non-agglutinogenic » vaccination was obtained for heifers with *Brucella abortus* strain 19, administered by the conjunctival route (Fensterbank and Plommet, 1979); the serological response to the vaccination lasts less than four months after both primary vaccination and after recall. The protection afforded is better than that afforded by subcutaneous normal vaccination, on condition that there is a recall, because this greatly increases the primary immunity (Plommet and Plommet, 1975).

The aim of this experiment was to study whether two conjunctival administrations of Rev.1 to ewes six months apart, could achieve a short post-vaccinal serological response and a protection as good as that achieved with subcutaneous normal vaccination with Rev.1.

Materials and Methods

1. Animals

Sixty ewes of Préalpes × Lacaune and Berrichonne breeds born in the brucellosis-free flock of the station, were used in this experiment. Fifty were born in December 1979: ten of these were kept as controls (group A), ten were vaccinated subcutaneously with 1.4×10^9 Rev.1 (group B) and thirty by conjunctival route (two drops of 30 µl) with 1.4×10^8 Rev.1 (group C). These forty animals were vaccinated at the age of four months (April 1980). The Rev.1 vaccine strain, originally obtained from Dr S.S. Elberg (University of California, Berkeley) was kept lyophilized and used after suspension in buffered saline. The count of viable organisms was done on day of inoculation.

Ten other ewes, born in April 1980 in the same flock, were vaccinated subcutaneously with 2.7×10^9 Rev.1 at the age of four months to complete the group B to twenty animals (October 1980).

The sixty ewes were inseminated on September 3rd, November 19th and December 4th, 1980. Forty-three lambed, fourteen were non-pregnant and three died.

Ewes from group C received a recall by conjunctival route, six months after primary vaccination (October 1980) with 2.9×10^8 Rev.1. At that time eighteen were fifty-six days in pregnancy.

The remaining fifty seven ewes were re-

inseminated (June 30th, 1981) and forty-two, recognized as pregnant, were kept for the experiment: eight of group A (four of each breed), seventeen of group B (three Berrichon and fourteen Préalpes × Lacaune) and seventeen of group C (two Berrichon and fifteen Préalpes × Lacaune). The ewes were introduced into an isolated sheep barn in four boxes: two of ten animals and two of eleven, containing non-different proportion of the three groups. They were challenged on the seventy-eighth day of pregnancy with 5×10^7 *Brucella melitensis* strain 53H38. The challenge strain was grown on trypticase soy agar for 24 h. A suspension was prepared in buffered saline and two drops of 30 µl were placed on the conjunctiva of each ewe. The count of viable organisms was done on day of challenge.

2. Examination procedures

The ewes were bled thirteen times from vaccination until challenge: the day of vaccination, two and four weeks later, then every two months. Then they were bled the day of challenge and, later, every two weeks until slaughter. Sera were submitted on microtiter to agglutination and complement fixation tests, according to the method described by Renoux *et al.* (1971), and to Rose Bengal plate test.

Cultures were made from vaginal excretion, samples were taken with swabs from challenge until parturition, and from uterine discharges on the day of parturition and the day after.

Aborted fetuses and dead lambs were autopsied and cultures were made from stomach content, spleen and lungs.

The ewes were slaughtered and necropsied forty-five days, on average, after parturition. Portions of udder, spleen, uterus, kidneys and liver were removed, as were submaxillary, parotid retropharyngeal, supramammary iliac, precrucial and prescapular lymph nodes. Portions of organs were ground either separately (spleen, uterus, udder) or in mixture (kidneys and liver) in a mixer (Kenwood, Woking). Lymph nodes were ground either by pairs (prescapular, precrucial, supramammary) or in mixture (iliac, nodes of the head) in a Stomacher (Prolabo, Paris). Tissue homogenates were seeded onto two plates. All cultures were done on Farrell's medium (Farrell, 1974) and colonies of brucella were enumerated.

3. Statistical

Comparisons were made by the Chi square test or by analysis of variance.

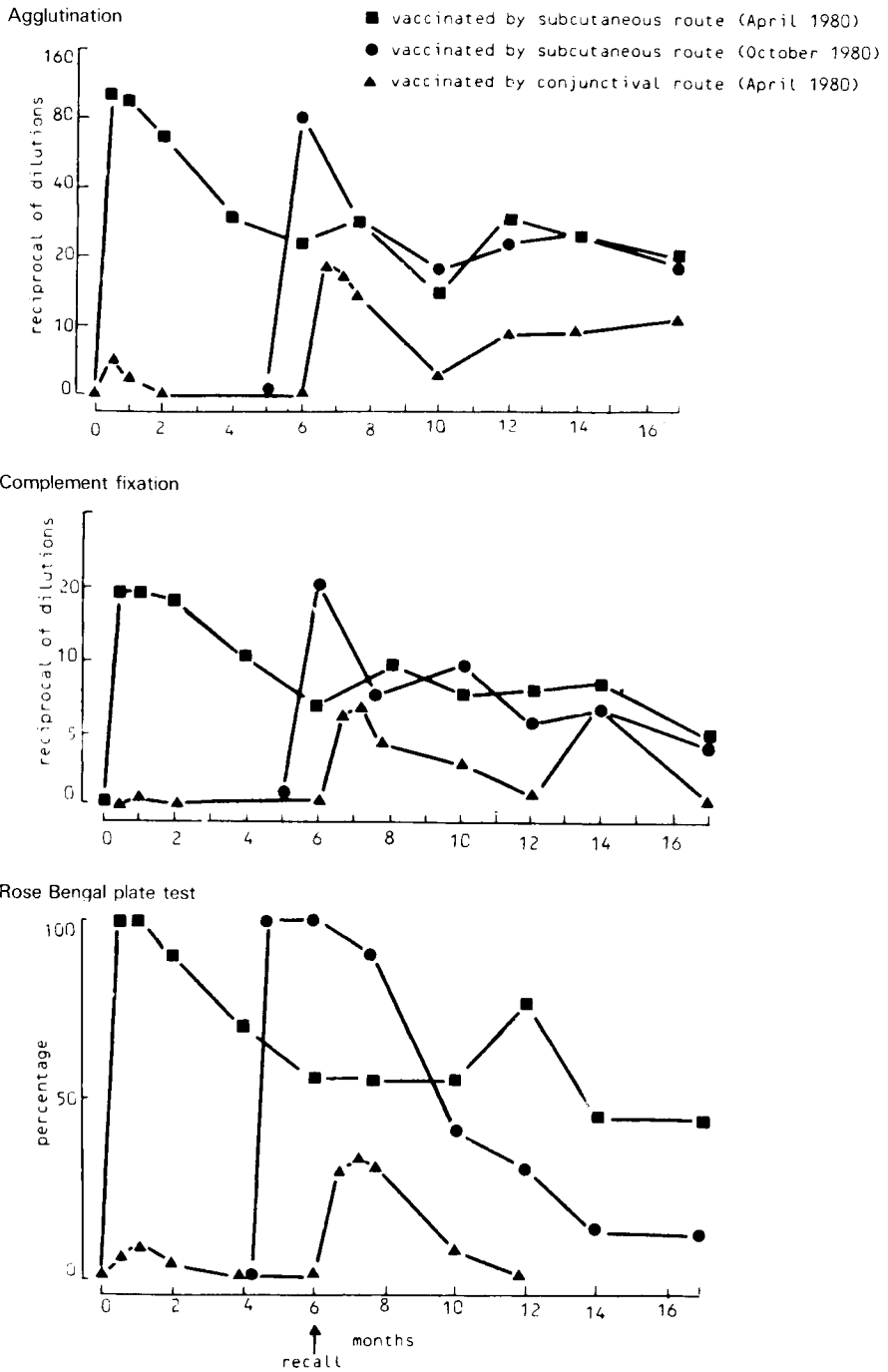


Fig. 1. — Evolution of antibody titers after vaccination

Results

1. Serological response after vaccination

After subcutaneous vaccination, all animals showed a rapid increase in antibody titers, as evidenced by the three serological tests (fig. 1a,b,c). Titers decreased with time, without significant difference between breeds or dose of vaccine, 1.4 or 2.7×10^9 organisms. Among the seventeen ewes kept for challenge and vaccinated subcutaneously seventeen or thirteen months earlier, thirteen were still showing agglutination antibodies, seven complement fixing antibodies and five a positive reaction with the Rose Bengal antigen.

After primary conjunctival vaccination, only 10 % of the animals showed a weak and short serological response: four months later the results were again negative for the tests. The conjunctival recall gave a stronger response: 28 ewes out of 32 (87.5 %) showed agglutinating and complement-fixing antibodies and 10 (33 %) a positive reaction with the Rose Bengal antigen. Among the 17 animals kept for challenge, 9 were still showing agglutinins and one complement fixing antibodies, 11 months after the recall, but all were negative and remained so with the Rose Bengal plate test as soon as 6 months after the recall.

2. Serological response after challenge

All animals showed agglutinating and complement-fixing antibodies as soon as two weeks after challenge, with titers increasing until the 8th-10th week. With the Rose Bengal plate test all animals of group B (subcutaneous route) and only 9 out of 16 of group C (conjunctival route) showed regular positive reaction in the course of the experiment, 7 remaining constantly negative.

3. Clinical results

No abortions occurred among the 18 pregnant ewes recalled with Rev.1 by conjunctival route on the 56th day of their first gestation.

After challenge, during the second gestation, the proportion of birth at term was higher in vaccinated animals than in controls ($P < 0.01$) as well as the proportion of viable lambs ($P < 0.001$) and the average duration of pregnancy ($P < 0.001$). In contrast, there was no significant difference between results in groups B and C, but there was a trend to better results in group C (table 1).

One ewe of group C was non-pregnant and another, of the same group, died of listeric encephalitis, 21 days after challenge.

4. Bacteriological results (table 2)

No excretion of the vaccinal strain Rev.1 was observed at the first parturition on the ewes recalled by conjunctival route during pregnancy or before insemination.

After challenge with *B. melitensis* strain H38 *ante-partum* vaginal excretion was observed on 23 ewes, one of which was the non-pregnant one. This excretion appeared as soon as one month after challenge. It was scarce on some animals: only one or very few colonies were isolated. On others by contrast, it was almost as abundant as after an abortion. It was observed once or several times on the same animal, both continuously and not. Seven ewes, after having excreted before parturition, gave birth to live lambs without any excretion *post-partum*.

After parturition, excretion was observed on 24 ewes, both in the cases of abortion and of clinically normal parturition. However, four ewes which gave birth to viable lambs at term

Table 1. - Clinical results

	Groups		
	A	B	C
Number of abortions/total	7/8	6/17	4/15 ^a
Duration of pregnancy average in days (limits)	120.4 (108-142)	140.4 (116-151)	142.1 (118-149)
Number of viable lambs/total (%)	1/14 (7.1)	15/26 (57.7)	16/26 (61.5)

a : one ewe died and one was non-pregnant.

Table 2. - Bacteriological results

	Number of isolation/total examined in groups		
	A	B	C
<i>Vaginal excretion</i>			
ante-partum	6/8	10/17	6/16 ^a
post-partum	8/8	10/16 ^b	6/15 ^c
<i>Presence of brucella in</i>			
fetuses	7/7	6/ 8 ^d	6/ 6
carcasses	7/8	4/17	4/16

a : one ewe was dead.

b : no samples were taken from one ewe.

c : one ewe died and another was non-pregnant.

d : two lambs died at birth because of dystocia.

Table 3. - Distribution of the infection. The infection was more generalized in controls (group A) than in vaccinated ewes (groups B and C)

	Number of isolations of <i>B. melitensis</i> (%) in groups		
	A	B	C
Number of animals	8	17	16
<i>Lymph-nodes</i>			
Of the head	6 (75)	1 (5.9)	1 (6.3)
Iliac	5 (62.5)	3 (17.6)	3 (18.8)
Supramammary	4 (50)	4 (23.5)	1 (6.3)
Precrural	3 (37.5)	0	0
Prescapular	3 (37.5)	0	0
<i>Organs</i>			
Udder	3 (37.5)	1 (5.9)	0
Uterus	3 (37.5)	0	0
Spleen	1 (12.5)	1 (5.9)	0
Kidneys and liver	1 (12.5)	0	0

Table 4. - Degree of infection. More organs and lymph nodes were heavily infected in controls than in vaccinated ewes

Degree of infection	Number of infected samples (%) in groups		
	A	B	C
less than 5 colonies per plate	12 (16.7)	3 (2.0)	4 (2.8)
from 6 to 25	6 (8.3)	2 (1.3)	0
from 26 to 125	8 (11.1)	4 (2.6)	1 (0.7)
from 126 to 525	2 (2.8)	1 (0.7)	0
more than 526	1 (1.4)	0	0
no isolation occurring	43 (59.7)	143 (93.5)	137 (96.5)

excreted very few *B. melitensis* as was observed by the reduced number of colonies on the plates.

All aborted fetuses were highly infected. Two lambs born at term, though dead because of dystocia, as well as the fetus of the ewe which died because of listeriosis, were not infected (table 2).

At slaughter the proportion of infected carcasses was much greater in control than in vaccinated animals ($P < 0.001$) as were the spread (table 3) and level (table 4) of infection. Brucella were recovered only from the lymph nodes of the head of the ewe which had died from listeriosis 21 days after challenge, but notably, none were recovered from its fetus. No brucella were recovered from ten ewes having excreted after parturition, three of which had aborted.

The ewes which, simultaneously, did not abort, did not excrete after parturition and did not shed *B. melitensis* at slaughter, were considered as protected. So the rates of protection were: in the control group A, 0/10, in the group B, 7/17 (41.2 %) and in the group C, 8/15 (53.3 %).

Discussion

From three tests we found that after subcutaneous vaccination with Rev.1 the serological response was still significant in a large proportion of animals at time of challenge, 17 months later. This foreshadows a probable continuation of the response in some animals. We had already observed this long-lasting response in the fields (unpublished data). This fact represents a serious drawback for a program of control of the disease by vaccination, since a serological screening is absolutely necessary to ensure that all animals are negative, so that vaccination can be ended.

After primary conjunctival vaccination, the serological response was weak and short by all tests: it lasted less than four months and this was only in some animals. In contrast, the response was stronger and longer lasting after the recall, contrary to its response in heifers where after recall it was scarcely more significant than after primary vaccination. However, ewes showed a negative serology as soon as six months with the Rose Bengal test, and 12 months with the complement fixation test after recall. Here there is a great advantage, which is made even greater by the fact that the protection afforded by conjunctival vaccination is at least equal to and even somewhat better than that provided by subcutaneous vaccination.

Other methods have been studied or need to be studied in order to find a way to reduce post-vaccinal titer while still maintaining the protective activity. Alton (1970) proposed to reduce the dose of Rev.1 in order to avoid abortions and mammary excretion of the vaccine strain in pregnant goats. At a dose of 5×10^4 cells, Rev.1 did not cause abortion, was not excreted in the milk and did not interfere with serological tests carried out after vaccination. But at this level of vaccination, only one out of ten vaccinated goats could resist to a challenge of 1.6×10^7 organisms, a challenge of the same size as ours. Because of the low degree of protection afforded, this process cannot be recommended.

In a previous experiment (unpublished data), we tried doses from 10^6 to 10^9 by conjunctival route, with a single dose of 10^6 for the recall. With two doses of 10^6 no protection was observed. With a primary vaccination of 10^7 plus a recall with 10^6 , the protection afforded was noticeable, with a very weak serological response, but because of the reduced number of animals in the experiment it was not possible for us to comment more precisely on the quality of this protection. After a primary vaccination with 10^8 or 10^9 and a recall with 10^6 the protection seemed to be good, with a weak serological response.

However, the need for two administrations of Rev.1 seems to be a major obstacle for sheep breeders. Research should be directed towards a single administration of a high dose of Rev.1 (10^9 for example) by conjunctival route.

It seems that nobody has ever observed any vaginal excretion of brucella before term in ewes. In heifers, Philippon *et al.* (1970) found that vaginal excretion between challenge and parturition is often weak and discontinuous and bears no relation to subsequent abortion or normal parturition, excretion at parturition and even pregnancy, since it was also observed in non-pregnant heifers. The same general characteristics were observed in ewes, but on some animals, unlike in heifers, the excretion was extremely abundant and it is possible that this plays a role in the spread of the disease.

Another interesting feature observed here was the spontaneous cure of a certain number of ewes. From six animals which excreted before term, no brucella were isolated at parturition or at slaughter, and ten others, which excreted at lambing, did not shed brucella at slaughter, six weeks later.

The administration of vaccine by conjunctival route is easy and reliable. In normal sheep

farming, ewes at the age of receiving the recall (i.e. 10 months) have often been mated, and many are already pregnant. We saw here that the recall could be administered with safety since no abortion or excretion of the vaccinal strain occurred among our animals. However, it would be by far more acceptable for farmers to have to do only one conjunctival administration instead of two. Research in progress should include investigation of this point, in particular in regard to the dose.

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Summary

In areas where the prevalence of *Brucella melitensis* infection in sheep is high, control of the disease cannot be led without the help of vaccination. The use of *Brucella melitensis* strain Rev.1 vaccine affords a very good protection. The drawback is that this vaccine, giving rise to long-lasting antibody titers, may disturb serological screening when a return is made to a test and slaughter policy, after several years of vaccination. There is a great need for another mode of vaccination which would avoid this disadvantage.

In this experiment, the potency of a vaccination by conjunctival route with 1.4×10^8 Rev.1, plus a recall of 2.9×10^8 six months later, was compared with that of the normal subcutaneous vaccination with 1.4 or 2.7×10^9 Rev.1.

The vaccination by conjunctival route afforded a somewhat better protection than the subcutaneous one, and with a shorter serological response. It may be possible that a single conjunctival vaccination, avoiding the difficulties of vaccinating twice in ovine flocks and giving few antibodies, could also afford a good protection. Research in progress should include investigation of this point.

References

- ALTON G.G., 1970. Vaccination of goats with reduced doses of Rev.1 *Brucella melitensis* vaccine. *Res. Vet. Sci.*, **11**, 54-58.
- ALTON G.G., ELBERG S.S., 1967. Rev.1 *Brucella melitensis* vaccine. A review of ten years of study. *Vet. Bull.*, **37**, 793-800.
- ELBERG S.S., 1981. Rev.1 *Brucella melitensis* vaccine. Part II 1968-1980. *Vet. Bull.*, **51**, 67-73.
- FARRELL I.D., 1974. The development of a new selective medium for the isolation of *Brucella abortus* from contaminated sources. *Res. Vet. Sci.*, **16**, 280-286.
- FENSTERBANK R., PLOMMET M., 1979. Vaccination against bovine brucellosis with a low dose of strain 19 administered by the conjunctival route. IV. Comparison between two methods of vaccination. *Ann. Rech. Vét.*, **10**, 131-139.
- PHILIPPON A., RENOUX G., PLOMMET M., 1970. Excrétion vaginale de *Brucella abortus* avant et après la mise bas. *Ann. Rech. Vét.*, **1**, 215-224.
- PLOMMET M., PLOMMET A.M., 1975. Vaccination against bovine brucellosis with a low dose of strain 19 administered by the conjunctival route. 1. Protection demonstrated in guinea-pigs. *Ann. Rech. Vét.*, **6**, 345-356.
- RENOUX G., PLOMMET M., PHILIPPON A., 1971. Microréaction d'agglutination et de fixation du complément pour le diagnostic des brucelloses. *Ann. Rech. Vét.*, **2**, 263-269.