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CONJUNCTIVAL VACCINATION OF YOUNG GOATS WITH *BRUCELLA MELITENSIS* STRAIN REV 1

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Résumé

VACCINATION CONJONCTIVALE DES CHEVRETTES AVEC *BRUCELLA MELITENSIS* SOUCHE REV 1. — Trois groupes de 15 chevrettes âgées de 4 mois ont été vaccinés avec *Brucella melitensis* Rev 1, deux par voie conjonctivale avec respectivement $1,1 \times 10^7$ et $1,1 \times 10^9$ UFC, le troisième par voie sous-cutanée avec $1,1 \times 10^9$. Quinze chevrettes non vaccinées constituaient le groupe témoin. Les 60 chevrettes ont été saillies à l'âge de 9 mois et demi. Trois mois plus tard, elles ont été éprouvées vis-à-vis de *B. melitensis* H38 administrée par voie conjonctivale à la dose de $5,1 \times 10^6$ UFC. La plupart des chevrettes vaccinées par voie conjonctivale ont présenté une réponse sérologique positive à l'épreuve au Rose Bengale et au test de fixation du complément ; toutes sont cependant devenues négatives en moins de 4 mois alors que certains animaux étaient encore positifs 8 mois après la vaccination sous-cutanée. Les réactions allergiques consécutives à la vaccination, quoique plus nombreuses dans le groupe des chevrettes vaccinées par voie sous-cutanée, affectaient encore quelques animaux 7 mois après la vaccination conjonctivale. La protection conférée par la vaccination conjonctivale avec $1,1 \times 10^9$ UFC Rev 1, évaluée par des critères cliniques et bactériologiques, s'est révélée légèrement supérieure à celle induite par $1,1 \times 10^7$ UFC et légèrement inférieure à celle résultant de la vaccination sous-cutanée, mais les différences ne sont pas significatives. Ces résultats, similaires à ceux antérieurement rapportés chez la brebis, confirment l'intérêt, chez la chèvre, de la vaccination conjonctivale.

Brucella melitensis infection typically affects sheep and goats which act as the reservoir of the disease also transmissible to cattle and man.

Control of the disease in small ruminants is mainly based on test and slaughter procedures and on vaccination. Eradication by identifying infected herds and slaughtering all the animals is only feasible in countries where prevalence is low, herds under close control and financial resources available. Where prevalence is high, as in most countries concerned with *B. melitensis* infection, vaccination alone as a preliminary, or vaccination combined with test and slaughter are more reliable strategies for the control of brucellosis in sheep and goats.

Brucella melitensis Rev 1 strain has proved to be the most effective vaccine for small ruminants (Alton and Elberg 1967, Elberg 1981, Alton 1985). The widely used dose of 1×10^9 viable organisms injected subcutaneously in young animals is generally recognized to produce a long-lasting protection, eg at least five years in goats (Alton 1968).

Subcutaneous vaccination with Rev 1 performed at 4 months of age induces a rapid rise in serological titres as measured by Rose Bengal Plate Test (RBPT) and Complement Fixation Test (CFT).

Titres then decrease and become negative in most animals by 6 months after vaccination. However, some more persistent CFT or RBPT titres have been reported in vaccinated lambs (Alton *et al* 1984).

Significant reduction of the serological response can be obtained by using reduced doses of Rev 1, eg 5×10^4 which in some experiments appeared to protect goats almost as well as the full dose (Alton 1970). Since this procedure has not been widely used for field vaccination, actual protection afforded by such low doses remains questionable.

An alternative method of reducing the serological response is the vaccination by the conjunctival route. It was previously shown in ewes that the dose of 1.4×10^9 CFU Rev 1 administered by the conjunctival route at 4 months of age induced a protection almost as good as that given by subcutaneous route, but with serological titres disappearing in all animals by 4 months after vaccination (Fensterbank *et al* 1985).

A vaccinal procedure giving a very short-lasting serological response is particularly needed in goats which usually first kid at 10-12 months of age. The subcutaneous vaccination is not convenient for dairy goats since it is likely to give titres lasting in some animals at the time of kidding and after. The

SC group (subcutaneous vaccination with 1.1×10^9 CFU Rev 1)

Goat	kidding ^a (no. of kids)	duration of pregnancy (days)	culture from ^b			degree of infection ^c at slaughter
			milk	vaginal swabs	dead kids	
80	A (1)	135	+	+	+	17
96	A (2)	144	+	+	+,+	34
138	A (2)	131	-	-	-, -	0
83	N (1)	153	-	-	...	1
89	N (2)	149	-	-	...	15
95	N (1)	153	-	-	...	0
110	N (1)	151	-	-	...	2
123	N (1)	156	-	-	...	1
136	N (2)	149	-	-	...	0
144	N (2)	155	-	-	...	3
185	N (2)	155	-	-	...	0
101	NP	0

CR9 group (conjunctival vaccination with 1.1×10^9 CFU Rev 1)

Goat	kidding ^a (no. of kids)	duration of pregnancy (days)	culture from ^b			degree of infection ^c at slaughter
			milk	vaginal swabs	dead kids	
81	A (3)	136	s	s	s,s,s	15
87	A (1)	111	-	-	-	17
107	A (3)	125	s	-	s, -, -	3
135	A (1)	136	+	+	+	32
91	N (2)	149	+	-	s ^d	44
92	N (2)	151	-	-	...	5
106	SB (3)	151	-	-	-, -, -	0
125	N (2)	148	s	-	...	10
127	N (2)	156	-	-	...	0
181	N (1)	149	-	-	...	0
182	N (1)	153	-	-	...	18
82	NP	7
124	NP	0
143	NP	0

a : A, abortion ; N, normal kidding ; SB, stillbirth ; NP, not pregnant

b : -, no growth ; +, heavy growth (more than 1 000 colonies per plate) ; s, slight growth (less than 10 colonies per plate) ; ND, not done

present experiment was therefore devised to test whether the conjunctival vaccination with Rev 1 in goats would both reduce the subsequent serological response and afford a reliable protection, as previously shown in sheep (Fensterbank *et al* 1985).

Materials and Methods

1. Bacterial cultures

The batch of Rev 1 vaccine used was freshly prepared from a vial of a freeze-dried seed culture of *B. melitensis*

strain Rev 1 maintained at Nouzilly. Challenge strain *B. melitensis* H38 was also kept freeze-dried in the *Brucella* culture collection at Nouzilly.

For both strains, the freeze-dried culture was harvested in 1 ml saline (pH 6.8). The resulting suspension was checked for purity and colonial morphology by streaking onto Trypcase Soy Agar (BioMérieux, Marcy l'Etoile, France) supplemented with 1 g/l yeast extract (Difco laboratories, Detroit, Mich, USA) (TSAYE medium). Smooth colonies were picked and grown on TSAYE medium slopes for 24 hours at 37 °C. The culture was then harvested in saline and diluted to the required concentration according to an absorbance reference curve (Spectronic 88 spectrophotometer, Bauch and

Bacteriological results

CR7 group (conjunctival vaccination with 1.1×10^7 CFU Rev 1)

Goat	kidding ^a (no. of kids)	duration of pregnancy (days)	culture from ^b			degree of infection ^c at slaughter
			milk	vaginal swabs	dead kids	
111	A (2)	124	+	+	+,+	36
113	A (1)	122	+	+	+	40
140	A (3)	138	+	+	+,+,+	39
84	N (3)	150	-	-	- ^d	1
97	N (2)	151	-	-	- ^d	0
115	N (2)	151	-	-	- ^d	0
118	N (3)	155	-	-	- ^d	0
102	NP	2
116	NP	6
128	NP	3
134	NP	0

Control group

Goat	kidding ^a (no. of kids)	duration of pregnancy (days)	culture from ^b			degree of infection ^c at slaughter
			milk	vaginal swabs	dead kids	
90	A (4)	115	+	+	+,+,+,+	41
94	A (3)	115	+	+	+,+,+	39
98	A (2)	112	ND	ND	+,+	44
99	A (2)	119	+	+	+,+	30
114	A (4)	124	+	+	s,s,s,s	35
132	A (1)	148	s	-	-	4
183	A (2)	114	+	+	+,+	40
186	A (2)	120	+	+	+,+	36
88	NP	8
139	NP	4

c: the degree of infection for each animal is the sum of the marks recorded as follows for 9 specimens of single or pooled tissues: 0, no Brucella; 1, 1-5 colonies; 2, 6-25; 3, 26-125; 4, 126-625; 5, more than 625
d: one kid dead within 48 h after birth

Lomb Inc, Rochester, NY, USA) (Wave length 600 nm). Actual concentrations used were measured by viable counts on TSAyE medium.

2. Animals and experimental design

Sixty three-week old female kids were purchased from three brucellosis-free flocks. They were negative to successive serological tests prior to vaccination.

When 4 months old, they were randomly allotted to four groups: 15 were vaccinated subcutaneously with 1.1×10^9 CFU Rev 1 (SC group), 30 were vaccinated by the conjunctival route, of which 15 with 1.1×10^9 CFU Rev 1 (CR9 group) and 15 with 1.1×10^7 CFU (CR7 group) and 15 remained unvaccinated as controls.

Goats were mated after oestrus synchronization at 9.5 months of age. Eleven goats were discarded for disease

or non pregnancy. The remaining 49 expected pregnant goats were moved to an isolation unit and randomly distributed into 8 pens.

They were challenged by the conjunctival route with one 33 μ l drop containing 5.1×10^8 CFU *B. melitensis* strain H38 when 88-89 days pregnant, ie at the age of 12.5 months and 8.5 months after vaccination.

Two goats died accidentally just after challenge. It therefore remained 47 goats for clinical and bacteriological examinations.

Goats were slaughtered 4 to 6 weeks after delivery.

3. Diagnostic procedures

Serological tests

Blood samples were collected at the time of vaccination, fortnightly for two months and thereafter monthly

until challenge. After challenge, goats were bled fortnightly until they were slaughtered. Sera were subjected to Rose Bengal Plate Test (RBPT) (Benga test, Iffa Mérieux, Lyon, France) and to Complement Fixation Test (CFT) (Brucella antigen CFT, BioMérieux, Marcy l'Étoile, France). Sera were considered as positive when fixation occurred at a dilution of 1/5 or higher.

Allergic tests

Goats were twice subjected to an allergic test, ie 7 months after vaccination and one month after challenge. One hundred microgrammes of brucellin INRA (Fensterbank and Dubray 1980) in 0.5 ml buffered saline were injected subcutaneously at the lower eyelid. Side of injection of the allergen was randomized. Degree of

swelling was recorded two days later by several independent observers who did not know the side of injection.

Bacterial examinations

Solid specimens were treated in a stomacher (Pro-labo, Paris) before plating. All cultures were carried out on Farrell's selective solid medium (Farrell 1974) and plates were read after 4 and 10 days of incubation at 37 °C.

Vaginal swabs and milk were taken on the day of delivery and the day after. Swabs were directly smeared onto the surface of the medium. Milk was centrifuged and cream and sediment were separately plated with a sterile loop.

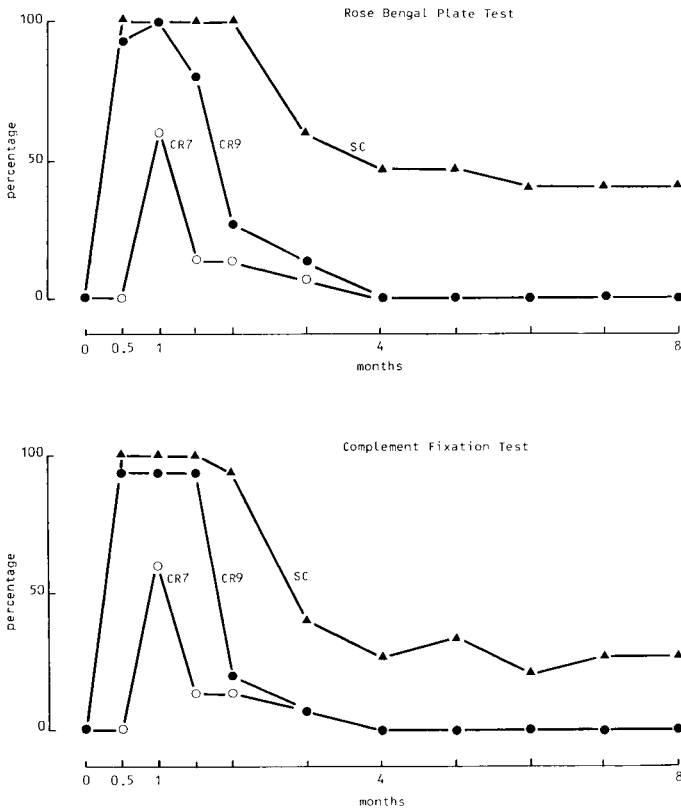


Fig 1. — Percentages of goats positive to Rose Bengal Plate test and to Complement Fixation test after vaccination with *B. melitensis* Rev 1.

Animals were considered as positive to CFT when fixation occurred at a dilution of 1/5 or higher.

SC : subcutaneous vaccination with 1.1×10^9 CFU Rev 1

CR9 : conjunctival vaccination with 1.1×10^9 CFU Rev 1

CR7 : conjunctival vaccination with 1.1×10^7 CFU Rev 1

There were 15 goats in each group.

Aborted foetuses or dead kids were necropsied and cultures were made from spleen, liver and stomach content.

As positive cultures from swabs, milk and kids were either very slight or heavy, results were recorded as: - (no growth), s (slight growth: less than 10 colonies per plate) and + (heavy growth: more than 1 000 colonies per plate).

From slaughtered goats, 9 specimens of single or pooled tissues were cultured, ie liver plus spleen, kidneys, uterus, udder as well as prescapular, precrural, iliac, supramammary and head (submaxillary + parotid + retropharyngeal) lymph nodes. Each specimen was cultured on two plates and the number of colonies per both plates were recorded as: 0, no *Brucella*; 1, 1-5 colonies; 2, 6-25; 3, 26-125; 4, 126-625; 5, more than 625. The degree of infection (DI) for each animal is the sum of the marks recorded for the 9 specimens (tables 1 and 2).

4. Statistics

Durations of pregnancy and degrees of infection were compared by the non-parametric U test of Mann and Whitney. Proportions of full-term kiddings, of excretors and of infected carcasses were compared by the chi-square test.

Results

1. Serological response to vaccination (fig 1)

Two weeks after subcutaneous vaccination, all animals were positive. Then titres and proportions of positive animals decreased, but 8 months after subcutaneous vaccination, 6/15 and 4/15 were still positive to RBPT and to CFT respectively.

Two weeks after conjunctival vaccination with 1.1×10^9 CFU Rev 1, 14/15 animals became positive. The 15th goat showed later on only a very low and transient response. However, all animals returned to a negative status in less than 4 months after vaccination.

After conjunctival vaccination with 1.1×10^7 CFU Rev 1, the response was delayed until one month, and only 9/15 became positive to one or both tests. All animals were negative four months after vaccination.

All controls remained negative until challenge.

2. Allergic response to vaccination and challenge

Seven months after vaccination, 13/15 goats in the SC group, 6/15 in the CR9 group and 1/15 in the CR7 group gave a positive reaction to brucellin whereas all controls were negative. One month after the challenge, 11/12 goats in the SC group, 11/12 in the CR9 group, 9/10 in the CR7 group and 6/8 controls showed a positive allergic response.

3. Clinical results (table 1)

No local or general reaction to vaccination was observed.

Ten goats were found not pregnant, ie 1 in the SC group, 3 in the CR9 group, 4 in the CR7 group and 2 in the control group. They were slaughtered 72 days after challenge.

Of the remaining 37 pregnant goats, 8/11 in the SC group, 7/11 in the CR9 group, 4/7 in the CR7 group and 0/8 controls kidded at term. All gave birth to viable kids, except goat 106 (CR9 group) which had 3 stillborn kids. Three kids died within 48 hours after birth: one from goat 91 (CR9 group), one from goat 84 and one from goat 115 (CR7 group).

4. Bacteriological results (table 1)

All isolates were *Brucella melitensis* strain H38 and vaccinal strain Rev 1 was never recovered.

Two out of 19 goats having kidded at term and 15/18 having aborted were excretors. Organisms were generally recovered in very large number (more than 1 000 colonies/plate) from swabs and milk. However, excretion was very scarce (<10 colonies/plate) in 4 goats: 81, 107 and 125 (CR9 group) and 132 (control).

Infection of aborted kids was generally heavy and related to excretion at kidding, except for goat 114 (control) which heavily excreted strain H38 whereas its four aborted kids were very slightly infected.

At slaughter, *Brucella* were recovered in 27/37 pregnant and in 6/10 non pregnant goats ie a total of 33/47 challenged animals.

All goats having excreted at kidding were found infected at slaughter.

Brucella were more often recovered from the head lymph nodes (26/23). In other tissues, occurrence in decreasing order was 22/33 for prescapular lymph nodes, 21/32 for the udder, 20/33 for the supramammary and precrural lymph nodes, 20/33 for uterus and spleen plus liver, 18/33 for the iliac lymph nodes and 17/33 for kidneys.

5. Statistical analysis of the data summarized in table 2

There was a significant difference in durations of pregnancy ($P < 0.001$), in degrees of infection at slaughter ($P < 0.01$), in proportions of full-term kiddings ($P < 0.01$), of excretors ($P < 0.02$) and of infected carcasses ($P < 0.05$) between control and vaccinated groups considered as a whole or separately. In contrast, no significant difference was observed between groups of vaccinated animals.

Table 2. - Summary of results

	Route of vaccination/dose			Control group
	Subcutaneous	Conjunctival		
		1.1×10^9	1.1×10^9	
Mean duration of pregnancy (days)	148	142	142	121
No. of pregnant goats	11	11	7	8
Full-term kiddings	8	7	4	0
Excretors ^a	2	5	3	7
Infected at slaughter (mean degree of infection) ^b	7 (6.6)	8 (13.1)	4 (16.6)	8 (33.6)

a : milk and/or vaginal swabs positive

b : the mean was calculated for all the animals in the group

Discussion

All kids conjunctivally vaccinated with Rev 1 became serologically negative by four months after vaccination. This result quite agrees with that previously recorded in lambs (Fensterbank *et al* 1985). It must be stressed, however, that this is not true in adult sheep and goats since titres were still observed in some animals one year after conjunctival vaccination with 1×10^9 Rev 1 (personal results). In practice, the conjunctival procedure applied to young animals allows to discriminate infected from vaccinated animals as early as 4 months after vaccination. On the contrary, the allergic test is not advisable as screening test in vaccinated flocks since vaccination, whatever the route, produces some long lasting reactions.

Regarding the protection, the three procedures of vaccination were shown effective against a challenge which led all controls to abort. Although no statistically significant difference was observed between vaccinated groups, protection appeared slightly higher in CR9 group than in CR7 one, and slightly lower than in SC one (table 2). Similar results were previously recorded in groups of lambs vaccinated subcutaneously with 1.6×10^9 and conjunctivally with 1.4×10^9 and 1.4×10^9 Rev 1 cells (Fensterbank *et al* 1985). In field conditions, abortion never concerns all females in an infected flock. It can therefore be assumed that the

immunity conferred to kids and lambs by a single 1×10^9 dose of Rev 1 administered either by subcutaneous or conjunctival route would practically be the same in conditions supposed less severe than the experimental ones.

Since it confers a suitable protection with a very short lasting serological response, the conjunctival vaccination of lambs and kids with a single dose of 1×10^9 Rev 1 is an advisable alternative to subcutaneous vaccination for control of the disease. Such a vaccination is of particular interest in areas where prevalence is low enough to allow combination of test and slaughter procedures and vaccination. Moreover, it is the only procedure which, leading all vaccinated goats to be serologically negative at the time of kidding, is not likely to hinder the marketing of milk production.

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Abstract

Three groups each of 15 four-month old female kids were vaccinated with *Brucella melitensis* strain Rev 1 either conjunctivally (1.1×10^7 or 1.1×10^9 CFU) or subcutaneously (1.1×10^9 CFU). One group of 15 kids remained unvaccinated as control. All kids were mated when 9.5 month old. Three months later, they were challenged conjunctivally with *B melitensis* strain H38 at a dose of 5.1×10^6 CFU. Blood samples collected before vaccination and throughout the experiment were subjected to Rose Bengal (RBPT) and Complement Fixation (CFT) tests. Allergy was tested both after vaccination and after chal-

lenge. Milk, uterine discharge as well as tissues from aborted foetuses and dead kids and from goats slaughtered 4-6 weeks after abortion or full-term kidding, were cultured on Farrell's medium. A serological response was shown in most kids vaccinated conjunctivally. However, 4 months after vaccination with either 1.1×10^7 or 1.1×10^9 CFU Rev 1, all animals were negative whereas some positive RBPT or CFT titres were still observed after the classical subcutaneous vaccination. There were more allergic reactions among subcutaneously than conjunctivally vaccinated animals, but sensitization was shown long-lasting in all vaccinated groups, excluding allergic testing as a screening method in vaccinated flocks. Against a challenge which led all controls to abort, conjunctival vaccination was slightly better with 1.1×10^9 than with 1.1×10^7 CFU and almost as effective than that given by the subcutaneous one. These differences however were not statistically significant. These results quite agree with those previously recorded for ewes. Since it confers a suitable protection with a very short-lasting serological response, the conjunctival vaccination of lambs and kids with a single dose of 1×10^9 Rev 1 is an advisable alternative to subcutaneous vaccination for the control of brucellosis in small ruminants. Moreover in goats, it is the only procedure which, leading to a rapid fall of post-vaccinal antibodies, is not likely to hinder the marketing of milk production subsequent to the first kidding.

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