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VACCINATION AGAINST BOVINE BRUCELLOSIS WITH A LOW DOSE OF STRAIN 19 ADMINISTERED BY THE CONJUNCTIVAL ROUTE. IV. COMPARISON BETWEEN TWO METHODS OF VACCINATION

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Résumé

VACCINATION CONTRE LA BRUCELLOSE BOVINE AVEC UNE DOSE REDUITE DE LA SOUCHE 19 ADMINISTREE PAR VOIE CONJONCTIVALE. IV. COMPARAISON ENTRE DEUX METHODES DE VACCINATION. — Vingt-deux génisses frisonnes, âgées de 6,5 à 9 mois, ont été vaccinées par voie sous-cutanée avec $11,5 \times 10^{10}$ CFU de *Brucella abortus* souche 19 et 22 autres avec $6,1 \times 10^9$ CFU par voie conjonctivale. Les 44 génisses ont reçu six mois plus tard un rappel de $5,7 \times 10^9$ CFU par voie conjonctivale. Avec 6 génisses non vaccinées, elles ont été inoculées au cours de leur première gestation avec $14,8 \times 10^6$ *B. abortus* souche 544.

Le rappel par voie conjonctivale après une vaccination primaire soit par voie sous-cutanée (mode S + C) ou par voie conjonctivale (mode C + C) n'a provoqué qu'une montée limitée en intensité et en durée des titres en agglutinines. La protection a été la même (36,4 %) dans les deux groupes mais, à l'abattage, les génisses vaccinées selon la méthode S + C étaient infectées à un degré moindre que celles vaccinées selon la méthode C + C.

Les données de cette expérience ont été regroupées avec celles d'une expérience précédente (Plommet et Fensterbank, 1976) afin de pouvoir juger de la protection sur 41 génisses vaccinées selon le mode C + C et sur 34 selon le mode S + C, par rapport à 12 génisses vaccinées par voie sous-cutanée selon la méthode standard et à 13 témoins. La meilleure protection a été observée après la vaccination selon la méthode S + C. La méthode C + C donne un résultat intermédiaire entre ceux donnés par la méthode S + C et la méthode standard. La méthode C + C devrait être réservée aux zones à faible taux d'infection brucellique. Ne donnant pratiquement pas d'anticorps, ce mode n'impose pas une restriction d'âge de vaccination comme la méthode sous-cutanée. Le mode S + C devrait être utilisé dans les zones fortement infectées. La vaccination primaire doit être faite entre 3 et 6 mois comme habituellement et le rappel 6 mois plus tard. En cas de danger, le rappel peut être fait n'importe quand, même sur les adultes. Le dépistage sérologique n'est perturbé que pendant les trois mois suivant le rappel.

Vaccination against bovine brucellosis with avirulent *Brucella abortus* strain 19 is usually performed on four to six months old heifers, so that the post-vaccinal response which lasts six to 12 months does not disturb the serological test on adults. It was stated (Manthei, 1968) that one subcutaneous vaccination would protect the cow for its entire life. This statement may however be questioned. We showed, in fact, that a booster administered by the conjunctival route, six months after the subcutaneous (standard) vaccination notably improved the immunity of the cow challenged by the standard method during its first pregnancy (Plommet and Fensterbank, 1976). Revaccination with an appropriate dose of 5×10^9 colony forming units (CFU) only induced a weak and short lasting serological response. Conjunctival revaccination may consequently be used to boost the immunity resulting from the primary subcutaneous vaccination in heifers, and even in adults.

We also demonstrated that two vaccinations by the conjunctival route six months apart induce a far better protection than a subcutaneous vaccination alone. We did not obtain significant differences between the heifers vaccinated either by the subcutaneous route plus a conjunctival revaccination (S + C) or by two successive conjunctival vaccinations (C + C), although the S + C vaccinated heifers seemed a little better protected than the C + C ones.

We decided to compare the two methods of

vaccination once more on a larger scale, using the same experimental design, so that the data from both experiments can be cumulated.

Material and methods

1. Animals experimental design

One hundred and thirty Friesian heifers, 6.5 to 9 months old, were bought from brucellosis free herds. They were divided at random into three groups, one of 11 non-vaccinated heifers, one of 40 receiving 11.5×10^{10} CFU *B. abortus* strain 19 by the subcutaneous (S) route and one of 79 receiving 6.1×10^9 CFU by the conjunctival (C) route.

The 119 vaccinated heifers were revaccinated six months later with 5.7×10^9 CFU by the conjunctival route.

After insemination, at 14 to 16 months of age, 50 pregnant heifers were taken at random, six from the non vaccinated group and 22 of each vaccinated group. When they were about six months in-calf, they were housed in an isolated cow shed described previously (Plommet *et al.*, 1970) and challenged with 14.8×10^8 *B. abortus* strain 544 by the conjunctival route.

Conjunctival vaccination was performed on the right eye and the challenge was given on the left one. Vaccinal and challenge suspensions were prepared from lyophilised culture grown on Albimi Agar (Pfizer). The growth was harvested and suspended in buffered

Table 1. — Clinical and bacteriological results after challenge in control group.

Animals	Clinical results		Bacteriological results			
	Birth ^(a)	Duration of pregnancy (days)	Brucella isolated from ^(b)			DI at slaughter ^(c)
			V	C	F	
462	A	229	+	+	+	6
470	P	263	+	+		0
471	A	240	+	+	+	19
475	T	281	+	+	+	11
481	A	232	+	+	+	27
586	T	278	—	—		0

^(a) Birth : T = at term (≥ 264 days, viable calf), P = premature (< 264 days, viable calf), A = abortion.

^(b) V = vaginal swab, C = colostrum, F = foetus.

^(c) DI = degree of infection, see table 4.

Table 2. — Clinical and bacteriological results after challenge in group of heifers vaccinated twice by conjunctival route.

Animals	Clinical results		Bacteriological results			
	Birth ^(a)	Duration of pregnancy (days)	Brucella isolated from ^(b)			DI at slaughter ^(c)
			V	C	F	
468	P	257	+	+		21
469	T	278	—	—		0
472	T	287	—	—		0
474	T	276	—	—		0
482	A	238	+	+	+	42
484	T	283	—	—		0
493	T	279	—	—		4
494	P	247	+	+		20
495	T	285	—	—		4
498	P	253	+	+		31
560	T	275	+	+		2
571	T	280	—	—		0
573	T	276	—	—		0
576	T	275	+	+		9
582	T	286	—	—		0
584	T	282	—	+		0
588	T	290	—	+		11
590	A	258	+	+	+	34
594	T	274	—	—		0
615	T	264	+	+		25
616	T	280	—	+		1
617	A	264	+	+	+	28

^a, ^b, ^c: see footnotes table 1.

Table 3. — Clinical and bacteriological results after challenge in group of heifers vaccinated by subcutaneous route then recalled by conjunctival route.

Animals	Clinical results		Bacteriological results			
	Birth ^(a)	Duration of pregnancy (days)	Brucella isolated from ^(b)			DI at slaughter ^(c)
			V	C	F	
465	T	284	—	—		0
467	T	281	—	—		0
478	T	277	—	—		0
479	T	278	+	+		7
486	A	234	+	+	+	12
487	T	280	+	+		0
488	A	210	+	+	+	15
489	T	282	—	—		0
492	T	269	—	+		11
499	T	269	—	—		6
565	T	272	—	—		0
566	T	275	—	—		2
569	T	274	+	+		6
575	A	246	+	+		24
585	T	281	—	—		0
587	T	280	—	—		0
589	T	274	+	+		0
608	T	282	+	—		0
609	T	277	—	—		1
611	T	277	—	—		0
614	T	281	—	+		2
620	T	279	—	—		1

^a, ^b, ^c: see footnotes table 1.

saline to the desired concentration. A viable count of each inoculum was made on Trypticase Soy Agar (Bio Mérieux).

2. Serological examinations

Heifers were bled before and at the time of vaccination, once a week for one month thereafter and then every month until the challenge. Animals were subsequently bled every two weeks following the challenge until the end of the experiment. Agglutination and complement fixation test were performed by the microtiter technique (Renoux *et al.*, 1971) comparatively with a standard serum (E.E.C. standard).

Results of agglutination were expressed either as international units (I.U./ml) or as the geometric mean of the titer, i.e. the order number of the greatest dilution resulting in a 50 % reaction (1 = 1/10, 2 = 1/20...) for a group of animals. An animal was considered as positive in serology if its agglutination was 80 I.U./ml, i.e. 100 % at a dilution of 1/40 and/or if its complement fixation titer was 1/5 or higher.

3. Clinical results

The parturitions were recorded as follows :
 T : full term calving (at least 264 days of pregnancy) with a living calf,
 P : premature calving (before 264 days) with a living calf,
 A : abortion, with dead foetus or calf surviving less than 48 hours.

4. Bacteriological examinations

At calving, cultures were performed from

vaginal swabs and colostrum, and from samples of spleen, lung and gastric content of aborted foetuses. Heifers were slaughtered about six weeks after parturition. Twenty-nine lymph nodes and portions of organs were taken, ground separately or in mixture, and pooled into 10 samples as indicated in table 4. Each sample was seeded onto two plates of WE medium (Renoux, 1954). After a one week 10 % CO₂ incubation at 37°C, the colonies were counted. The number of colonies per gram of sample was expressed as follows : 0 = no *Brucella*, 1 = less than 5, 2 = from 6 to 25, 3 = from 26 to 125, 4 = from 126 to 625 and 5 = more than 625. These numbers from 0 to 5 represent the degree of infection (DI) of each sample. The degree of infection of a cow is the sum of the DI of the ten samples.

The tissue samples were stored after culture at - 20 °C. When no *Brucella* were recovered, the sample was seeded again on five plates of Farrell's medium (1974), which has been shown by Farrell and Robertson (1972) to have an isolation rate comparable with guinea-pig inoculation and by our previous trials to be less inhibitory than the WE medium.

5. Statistical analysis

The Chi square test and analysis of variance were used.

Results

1. Serological response after vaccination

After primary subcutaneous vaccination,

Table 4. — Bacteriological data at post mortem examination in control group.

Animals	Number of <i>Brucella</i> isolated from ^(a)										D.I. ^(b)
	A	B	C	D	E	F	G	H	I	J	
462		1	1	3			1				6
470											0
471	4	1	2	5	2		1		4		19
475	4	1		3	2		1				11
481	5	4	5	5	3	1	2		2		27
586											0

^(a) Number of *Brucella* per gram of tissue. No indication = absence of *Brucella*, 1 = from 1 to 5 bacteria, 2 = from 6 to 25, 3 = from 26 to 125, 4 = from 126 to 625, 5 more than 625.

Samples : A = supramammary lymph nodes, B = iliac, C = submaxillary, parotid and retropharyngeal, D = quarters of udder, E = subiliac, F = spleen, G = prescapular, H = mediastinal, I = uterus, J = liver + kidneys.

^(b) D.I. : degree of infection = sum of the classes of infection of the ten samples.

Table 5. — Bacteriological data at post mortem examination in group of heifers vaccinated twice by conjunctival route.

Animals	Number of Brucella isolated from ^(a)										D.I. ^(b)
	A	B	C	D	E	F	G	H	I	J	
468	5	2		5	3			1	3	2	21
469											0
472											0
474											0
482	5	5	5	5	5	5	3	4	3	2	42
484											0
493									4		4
494	5	2	3	4	1	1	1	1	2		20
495									4		4
498	5	4	4	5	2	3	2	2	5	3	31
560		1							1		2
571											0
573											0
576	4			5							9
582											0
584											0
588	4	1		5			1				11
590	5	2	2	5	2	2	3	4	5	4	34
594											0
615	5	5	2	5	4	1		1	1	1	25
616							1				1
617	5	5	5	5	4	2	1		1		28

^{a, b} see footnotes table 4.

Table 6. - Bacteriological data at post mortem examination in group of heifers vaccinated by subcutaneous route then recalled by conjunctival route.

Animals	Number of Brucella isolated from ^(a)										D.I. ^(b)
	A	B	C	D	E	F	G	H	I	J	
465											0
467											0
478											0
479	1						1		5		7
486	4	1		4					3		12
487											0
488	4	3	1	3		1			3		15
489											0
492	5	2		4		0					11
499	1 1	2									6
565											0
566					2						2
569						1			5		6
575	5	4	3	5	2	1		2	2		24
585											0
587											0
589											0
608											0
609					1						1
611											0
614	2										2
620								1			1

^{a, b} : see footnotes table 6

Table 7. — Comparison of protection to challenge by two methods of vaccination.

Vaccination ^(a)	Isolation of Brucella						Mean D.I. ^(b)
	Number Animals	Full term calvings (%)	Mean duration of pregnancy (days)	Vaginal swabs	At slaughter	Total	
None	6	2 (33)	254.0	5	4	5	10.5
C + C	22	16 (73)	272.1	9	13	14	10.5
S + C	22	19 (86)	271.0	8	11	14	3.9

^(a) C + C : two successive conjunctival vaccinations ; S + C : subcutaneous vaccination plus conjunctival recall.

^(b) Average degree of infection.

Table 8. — Comparison of protection to challenge by three methods of vaccination (results of two successive experiments).

Vaccination ^(a)	Isolation of Brucella						Mean D.I. ^(b)
	Animals number	Full term calvings (%)	Mean duration of pregnancy (days)	Vaginal swabs	At slaughter	Total	
None	13	3 (23)	246.7	12	11	12	13.7
S	12	6 (50)	259.2	11	9	11	9.8
C + C	41	30 (73.2)	270.4	19	25	28	8.7
S + C	34	27/33 ^(c) (81.8)	270.4	14	16	20	3.7

^(a) S : subcutaneous (standard) vaccination ; C + C : two successive conjunctival vaccinations ; S + C : subcutaneous vaccination plus conjunctival recall.

^(b) Average degree of infection.

^(c) One cow had aborted but was not infected by Brucella.

the conjunctival revaccination produced a very weak, or no, increase in agglutination titers (fig. 1). Some heifers showed complement fixing (CF) antibodies, so that the proportion of positive animals increased from about 15 % before recall to 30 % afterwards (fig. 2). This increase in number of cattle with CF titers lasted about 12 weeks, after which the number of animals still positive was about the same as that usually observed after subcutaneous vaccination alone. All heifers were negative 58 weeks after primary vaccination.

Conjunctival vaccination and recall gave a mean agglutination response below 30 I.U./ml. About 10 % of the heifers showed CF antibodies after primary vaccination and 15 % after recall. Twelve weeks later, all heifers were negative.

2. Protection

Vaccinated heifers of both groups had significantly better full term calving rate ($P \leq 0.01$), durations of pregnancy ($P \leq 0.01$) and excreted less ($P \leq 0.05$) than controls. In all, 36 % of vaccinated heifers were completely protected against infection, i.e. no *Brucella* were found either at calving or at post mortem examination.

In each of the vaccinated groups, the clinical and bacteriological results were not very different and total protection was the same (tables 1 to 7). However, at post mortem examination, S + C vaccinated heifers were infected to a lower degree than C + C ones, which were infected to the same degree as controls. Two of six controls were found not infected at slaughter : the first (586), apart from one CF positive test after challenge, was never found infected by any examination ; the second (470) showed a strong serological response and excreted at premature calving, but no *Brucella* was isolated at post mortem examination.

Discussion

Calfhood subcutaneous vaccination with strain 19 provides major assistance in the prevention and control of bovine brucellosis. It has been shown to be of great assistance in reducing infection and permitting an economical eradication. However, this vaccination has two disadvantages :

1°) development and persistence of post-vaccinal antibodies,

2°) restriction of age vaccination to 3 to 6 month old calves in order to minimize the persistence of post-vaccinal titers. This restriction is often inconvenient or incompatible with husbandry and management practices of many cattlemen. Thus, the disadvantages have resulted in reduced levels or a total absence of vaccination, in spite of dangers of recontamination and of the need for vaccinated heifers for replacement in non brucellosis-free areas.

In order to reduce the persistence of post-vaccinal antibodies, the use of reduced doses of strain 19 by the subcutaneous route was proposed, residual titers decreasing with the reduction in the dose of vaccine injected. The efficiency of reduced doses has not been proven in experimental conditions until now but it was shown to be helpful in the field to clean up large herds and protect them from reinfection (Nicoletti, 1976).

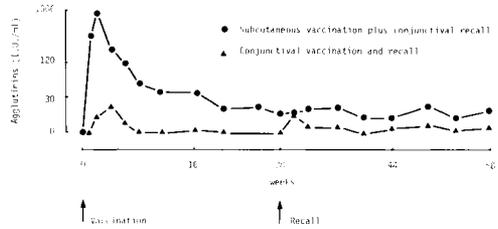


Fig. 1. — Geometric mean of agglutinins after primary vaccination and recall.

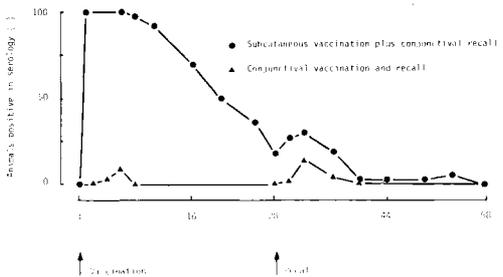


Fig. 2. — Rate of animals positive in serology after vaccination and recall. Animals were considered as positive in serology if their agglutination titer was ≥ 80 I.U./ml and/or if their complement fixation titer was $\geq 1/5$.

On the contrary, reduced doses of strain 19 administered by the conjunctival route, either as revaccination after subcutaneous vaccination or as two conjunctival vaccinations, produced low serological responses and good protection on guinea pigs (Plommet and Plommet, 1975) in heifers (Plommet and Fensterbank, 1976), and in adult cows (Nicoletti, Jones and Berman, 1978).

In this experiment, some heifers vaccinated by the subcutaneous route were somewhat too old. Had the primary vaccination been effected within 3 to 6 months of age, residual titers should have disappeared. Even with the delayed vaccination, the rate of animals positive in serology after revaccination quickly returned to the normal rate usually observed one year after single subcutaneous vaccination. C + C vaccination induces only a weak and transient antibody response, even in adult cows (unpublished data).

S + C vaccinated heifers were infected to a lesser degree at slaughter than the C + C ones. Other clinical and bacteriological results were also somewhat better in the S + C vaccinated group, but the number of cattle free of infection was the same in both groups. In this experiment, controls showed an unusually low average of infection, since two heifers were not infected at slaughter. Such results, although infrequent, are not aberrant. In our previous experiments, we already observed one non-infected control heifer out of 58 challenged during the first pregnancy with 15×10^6 *Brucella abortus* strain 544. We also observed that some heifers, showing high titers of antibodies after challenge and found infected at calving by isolation of *Brucella*, could not be demonstrated to be infected at slaughter, six weeks later (heifers 487, 584, 589 and 608 in this work).

As this experiment followed the same plan as the first one (Plommet and Fensterbank, 1976) and as results are statistically homogeneous in corresponding groups, in particular the degrees of infection at slaughter which do not differ (controls, $F = 1.47$; C + C vaccinated group, $F = 2.06$; S + C, $F = 0.02$ for $F \leq 4.08$ at the level of signification of $P = 0.05$), it is possible to add data of both experiments. Pooled results are shown on table 8.

In the first experiment, a subcutaneous (standard) vaccination gave poor protection; clinical results were better than in the control group ($P < 0.05$) but only one heifer out of 12 was completely protected. On the contrary,

the conjunctival revaccination after a primary vaccination either by the subcutaneous or conjunctival routes resulted in significant differences in the rates of full term calvings ($P \leq 0.001$), excretion at calving ($P \leq 0.001$) and complete protection ($P \leq 0.05$) in comparison with controls. The same differences were observed when S + C and C + C heifers were compared to subcutaneously vaccinated heifers, but they were less significant ($0.05 < P < 0.10$). At slaughter, S + C vaccinated heifers were infected to a lesser degree than controls ($P \leq 0.01$) and C + C vaccinated animals ($P \leq 0.05$).

Thus, the best protection is given by S + C vaccination. This is better than the C + C method which itself is better than subcutaneous standard vaccination. In our previous experiments, we regularly obtained poor protection with subcutaneous vaccination, although this method seems to give good results in the field (statistical studies of Jones and Berman, 1975 and of many others). However, we feel sure that field vaccination by C + C and S + C methods would provide better protection than subcutaneous vaccination alone, and would not disturb subsequent serological tests.

In conclusion, we recommend two schemes of vaccination, according to the prevalence of brucellosis. In areas with a low incidence of infection, the C + C vaccination should be used. Priming and recall vaccinations should be done six months apart, but the delay might be extended to 12 or shortened to four months to fit to the constraints of the breeding system. For example, the first vaccination could be done between three and nine months of age and the recall between nine and 15 months.

In heavily infected areas, the S + C method should be used to induce a higher level of immunity. Primary vaccination must be done, as usual, at three to six months of age. Secondary conjunctival vaccination should be done six months later on heifers or at any time, even on adult cows for animals at risk, to boost the basic immunity. Thus in previously subcutaneously vaccinated non infected herds where an epidemic of brucellosis occurs, the best way to keep the disease under control would be to revaccinate the whole herd by the conjunctival route and to cull the serologically positive animals. The serological tests could be done before and as soon as three months after conjunctival vaccination.

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Summary

Twenty-two Friesians heifers, 6.5 to 9 months old, were vaccinated subcutaneously with 11.5×10^{10} colony forming units (CFU) of *Brucella abortus* strain 19 and 22 others with 6.1×10^9 CFU by the conjunctival route. The 44 heifers were revaccinated six months later with 5.7×10^9 CFU by conjunctival route. Together with six non vaccinated heifers, they were challenged during their first pregnancy with 14.8×10^6 *B. abortus* strain 544 by the conjunctival route. The conjunctival recall after primary vaccination either by the subcutaneous route (S + C method) or by the conjunctival route (C + C method) gave a very weak and transient increase in agglutination titers. The protection was the same (36.4 %) in both groups, but at slaughter, there was less infection in the S + C vaccinated heifers than in the C + C animals. The data from this experiment were pooled with those of previous one (Plommet and Fensterbank, 1976) in order to evaluate 41 C + C and 34 S + C vaccinated animals, in comparison with 12 subcutaneously standard vaccinated heifers and 13 controls. The best protection was afforded by the S + C method. C + C vaccination resulted in protection intermediate between that of the S + C and the standard method. The C + C vaccination should be used in areas with a low prevalence of brucellosis. Since the serological response to C vaccination is weak and short-lasting, the age of vaccination is not critical as with the subcutaneous method. The S + C vaccination should be used in heavily infected areas. Primary vaccination must be performed as usual at three to six months of age, and revaccination six months later on heifers. For animals at risk, secondary vaccination may be carried out at any time, even on adult cows. Serological tests can be performed as soon as three months after conjunctival revaccination.

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