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VACCINATION AGAINST BOVINE BRUCELLOSIS WITH A LOW DOSE OF STRAIN 19 ADMINISTERED BY THE CONJUNCTIVAL ROUTE

I. — PROTECTION DEMONSTRATED IN GUINEA PIGS

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RÉSUMÉ

VACCINATION CONTRE LA BRUCELLOSE BOVINE AU MOYEN D'UNE FAIBLE DOSE DU VACCIN B19 ADMINISTRÉ PAR VOIE CONJONCTIVALE.

I. — PROTECTION TESTÉE SUR COCHONS D'INDE

Des cobayes ont reçu des doses variables du vaccin *Brucella abortus* B 19, soit par voie conjonctivale, soit par voie sous-cutanée, une fois ou deux fois à trois mois d'intervalle (fig. 1). Les réponses sérologiques en agglutinines ou anticorps fixant le complément ont été suivies dans certains lots sur 4 à 6 cobayes tirés au sort. La résistance conférée par la vaccination a été éprouvée par inoculation intramusculaire de $5 \cdot 10^8$ *B. abortus* 544, 3 mois après la vaccination primaire ou de rappel, puis recherche et dénombrement des *Brucella* dans la rate et les ganglions lymphatiques, 40 jours plus tard.

Les réponses sérologiques à la vaccination par voie conjonctivale aux doses de $1 \cdot 10^6$ et $1 \cdot 10^7$ bactéries vivantes sont faibles et transitoires, même après vaccination de rappel ; elles sont inférieures à celles résultant d'une vaccination par voie sous-cutanée à la dose de $1 \cdot 10^6$ bactéries et très inférieures à celles résultant de la vaccination normale d'épreuve, à la dose de $7 \cdot 10^9$ bactéries, par voie sous-cutanée.

La protection, exprimée par le pourcentage de cobayes trouvés non infectés à l'autopsie est de 40 p. 100 (tabl. 4) pour les lots recevant la dose normale d'épreuve par voie sous-cutanée. Si cette vaccination normale est suivie d'un rappel par voie conjonctivale à faible dose ($1 \cdot 10^6$ ou $1 \cdot 10^7$), la protection est de 57 p. 100. Deux vaccinations par voie conjonctivale confèrent, selon la dose ($1 \cdot 10^6$ à $1 \cdot 10^9$) une protection de 47 à 60 p. 100. Deux vaccinations à faible dose ($1 \cdot 10^6$) par voie sous-cutanée confèrent une protection de 75 p. 100. Tous les témoins sont infectés.

La vaccination par 2 instillations conjonctivales d'une dose de $1 \cdot 10^7$ bactéries n'induisant qu'une réponse sérologique négligeable, et donnant une protection au moins équivalente à celle de la vaccination standard, on en conclut qu'un système analogue serait avantageux pour la vaccination des Bovins.

Mots clés : *Anticorps*, *B19*, *Brucella abortus*, *cobaye*, *vaccination (voie conjonctivale)*.

In a previous experiment in pregnant cattle, we had observed that the injection of an antibiotic before the conjunctival inoculation of a 100 p. 100 infecting dose of the virulent culture *Brucella abortus* 544 completely modified the course of the infection. During the bacteriostatic phase immunity developed without significant serological reaction, which, after a phase of dissemination of infection, was sufficient to cause regression and total clearance of infection (PHILIPPON *et al.*, 1971).

This observation suggested that the conjunctival inoculation of a suitable dose of a less invasive *Brucella* culture might reproduce this dissociation between immune response and serological response. *B. abortus* vaccine strain B 19, of low virulence for cattle, normally administered at a high dose (9×10^{10} bacteria) by the subcutaneous route, could be used for this purpose.

The guinea pig is the experimental animal recommended for potency testing of the vaccine B 19. In contrast to the mouse (VERGER, 1971), the guinea pig is sensitive to conjunctival inoculation of *Brucella*: the 50 p. 100 infecting dose of strain 544 being of the order of 10^5 bacteria, compared with 10^2 by the intramuscular route. Conjunctival inoculation of strain B 19 should, by the colonization of the regional lymph nodes, induce some immunity. This immunity could be measured by the recommended method of intramuscular inoculation of guinea pigs with the virulent challenge strain *B. abortus* 544. (THORNTON and MUSKETT, 1972).

In this study, we have determined in the guinea pig the serological response to vaccination with strain B 19 given in different doses by the conjunctival route, and the protection conferred by this vaccination.

Three successive experiments were performed :

a) Experiment A determined which dose of vaccine, administered one or two times by the conjunctival route, was able to induce a suitable immunity without significant serological reactions. Two optimal combinations were defined which consisted of two instillations of either 10^6 or 10^7 bacteria at 3 month interval.

b) Experiment B determined the efficacy of two doses of 10^6 bacteria in comparison with :

1. The standard vaccination method for guinea pigs (7×10^8 bacteria by the subcutaneous route) ;
2. The standard vaccination followed by re-vaccination with 10^8 bacteria administered by the conjunctival route ;
3. Vaccination consisting of two subcutaneous injections of 10^8 bacteria at a 3 month interval.

c) Experiment C examined the second optimal combination (2 doses of 10^7 bacteria by the conjunctival route) in comparison with the standard vaccination method, with or without re-vaccination by the conjunctival route. The serological responses to the vaccination were not studied in this last experiment, because, in the meantime, this response had been determined in cattle. We have added, instead, a different comparison. In practice, a booster vaccination with vaccine 45/20 is often administered to cattle sometime after vaccination with B 19. We thought it would be useful to know the efficacy of this booster vaccination comparatively to the re-vaccination with B19 given by the conjunctival route.

MATERIALS AND METHODS

Animals

Guinea pigs of the Hartley Albino strain were 2 months of age and weighed about 350-400 g at the beginning of the experiments. Only males being available were used in Experiment A and only females in Experiments B and C.

Preparation of vaccine and challenge suspensions

The vaccine strain *B. abortus* B 19 came from National Animal Diseases Laboratory, Ames, U.S.A. The challenge strain *B. abortus* 544 came from Central Veterinary Laboratory, Weybridge, G. B. They were lyophilized and a new ampoule was taken for the preparation of the suspensions for each successive experiment.

The content of the ampoule was suspended in 3 drops of broth, spread on the surface of a slope of *Brucella agar Albimi* (PFIZER), incubated 48 h in an atmosphere of 10 p. 100 CO₂. After an intermediate transfer a 24 h culture on the same medium was suspended in buffered saline, standardized by photometric reading at 600 m μ , then diluted to obtain the desired concentration. A viable count of the inoculum was made by spreading a suitable dilution of the final suspension on 10 petri plates of *Trypticase soy agar* (BIOMERIEUX).

The 45/20 vaccine utilized was a commercial vaccine (Abortox, Merieux) consisting of a killed suspension of *B. abortus* rough strain 45/20 in an oil-in-water adjuvant. This vaccine was used for the booster vaccination at a dose of 10¹⁰ bacteria given subcutaneously.

Protocols of experiments

The official protocol for the control of the efficacy of vaccine B19 is as follows (THORNTON and MUSKETT, 1972) : vaccination by the subcutaneous route with a dose of 7×10^8 bacteria (this we refer to as the standard vaccination) ; 60 days later the challenge is given by the intramuscular injection of 5×10^8 bacteria of strain 544 ; 40 days later autopsy and search for *Brucella* in the spleen.

Our protocol differed from the above as follows :

- the interval between single vaccination and challenge inoculation was either 3 months (experiment A) or 6 months (experiments B and C) (fig. 1) ;
- groups given a second vaccination after a 3 month interval were challenged 3 months after the second vaccination ;
- at autopsy (40 days after challenge) the *Brucella* were looked for and enumerated in the spleen and in 8 lymph nodes ;
- blood samples were taken by cardiac puncture at variable intervals after vaccination from representative animals in some treatment groups ;
- vaccinations consisted of either one or two administrations of the doses and by the route appropriate for each experiment, as indicated in tables 1, 2 and 3.

The subcutaneous (vaccine) and intramuscular (challenge) injections were made in volumes of 0.2 ml. The conjunctival instillations were made by depositing 5 μ l on the eye by means of a micro-syringe of 25 μ l (Hamilton).

Enumeration of Brucella in the spleen and lymph nodes

At autopsy, the spleen and the following 8 lymph nodes or groups of lymph nodes were removed in a sterile manner : 1 or 2 iliac, 1 mesenteric, 2 cervical, 2 submaxillary, right and left axillary, right and left precural.

The spleen was trimmed to remove fat, weighed, emulsified in 4 times its weight of saline in a glass tissue grinder. From this suspension and from successive appropriate dilutions, 0.1 ml portions were spread on 2 petri plates of TSA medium, then incubated in an atmosphere of CO₂ for 5 to 8 days. The colonies of *Brucella* were counted on the petri plates which contained between 30 and 500 colonies, or less than 30 in the case of the original suspension which was a 1/5 dilution. The least number detectable was thus 50 *Brucella* per spleen. The number per spleen was expressed by its decimal log, with the value 0 representing absence of *Brucella*. The intensity of splenic infection per group of guinea pigs was expressed as the mean of these values.

The lymph nodes were trimmed to remove fat, flamed, minced with scissors, rubbed on the surface of petri plates in a manner to extract the liquid. After incubation, the colonies of *Brucella* were counted. The number was expressed according to the following logarithmic scale: 1 for 1 to 4 colonies; 2 for 5 to 24 colonies; 3 for 25 to 124 colonies; 4 for more than 124 colonies. The mean of the values for the 8 lymph nodes or groups of all the guinea pigs of the treatment group was the index of lymph node infection.

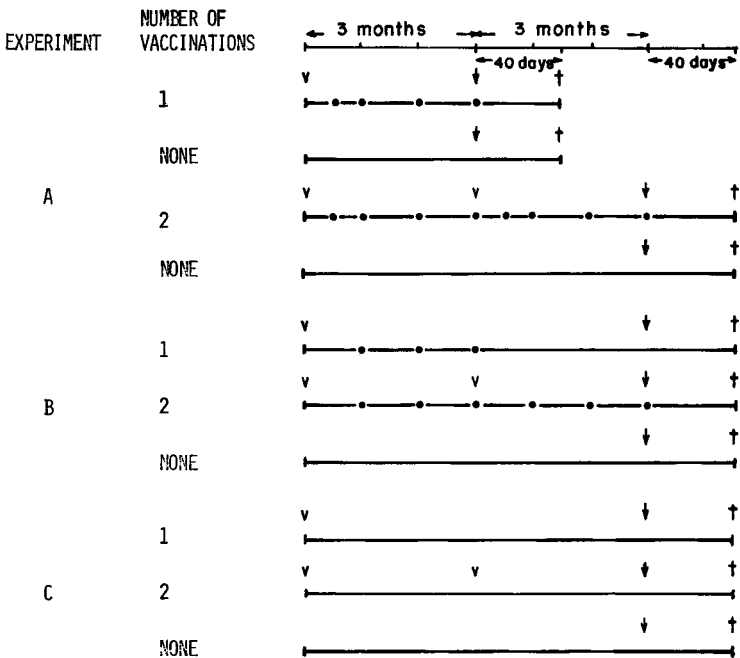


FIG. 1. — *Experimental design of the three experiments*

v indicates Vaccination; ↓: challenge; ↑: autopsy; ●: blood sampling

Schéma des 3 expériences

v indique la date de la Vaccination; ↓: l'inoculation; ↑: l'autopsie; ●: les prises de sang

Serological examinations

Four to six guinea pigs, chosen at random from each treatment group at various intervals after vaccination, were bled by cardiac puncture. The titers of agglutinins and complement fixing antibodies were determined by the Microtiter method described previously (RENOUX, PLOMMET and PHILIPPON, 1971), starting with a dilution of 1/5 and by comparison with the reference serum which has a titer of 1/640 in the two tests. The geometric means of the titers of the sera in each treatment group were plotted in the figures.

RESULTS

I. — *Experiment A : Choice of an optimal dose of vaccine for the conjunctival route*

Table 1 shows that, in comparison with the controls, a single vaccination by the conjunctival route with a dose of 10^6 bacteria did not confer any protection. The same dose by the subcutaneous route induced some immunity as shown by the reduced level of infection in spleen and lymph nodes. Higher doses given by the conjunctival route gave measurable protection, as shown by reduced infection (10^7) and by protection (10^8 and 10^9).

TABLE I

*Experiment A**Expérience A*

Vaccination			Protection ⁽²⁾	Intensity of infection ⁽³⁾	
Dose	Number	Route ⁽¹⁾ (Voie)		Spleen (Rate)	Lymph nodes (Ganglions)
10^6	1	C	0/8	5.6	2.7
10^7		C	0/8	2.8	1.1
10^8		C	4/9	1.7	0.7
10^9		C	3/10	2.3	0.6
10^6		SC	0/8	2.8	1.0
Control			0/11	5.2	2.6
10^6	2	C	6/8	0.9	0.1
10^7		C	4/6	1.1	0.3
10^8		C	8/10	0.5	0.1
10^9		C	4/10	1.5	0.1
Control			0/10	5.7	1.9

⁽¹⁾ C = conjunctival route ; SC = subcutaneous route.
(C = voie conjonctivale ; SC = voie sous-cutanée).

⁽²⁾ Guinea pigs protected/total. Protected = no *Brucella* isolated from any sample.
(Cobayes protégés/total. Protégés = absence de *Brucella* isolées d'aucun échantillon).

⁽³⁾ Average of the log of the total number of *Brucella* per spleen, and index of infection of the lymph nodes.

(Moyenne du log du nombre total de *Brucella* par rate, et index d'infection des ganglions lymphatiques).

Two vaccinations by the conjunctival route at 3 month interval, induced significant immunity even with the dose of 10^6 bacteria.

Figure 2 gives the evolution of anti-brucella antibodies after vaccination, each

point representing the mean titer of 4 sera. The agglutination reactions remained low, scarcely exceeding an average titer of 1/40 even after the second vaccination. The abnormal fluctuations observed are due, without doubt, to the small number of sera which were taken in order to limit accidents due to cardiac puncture.

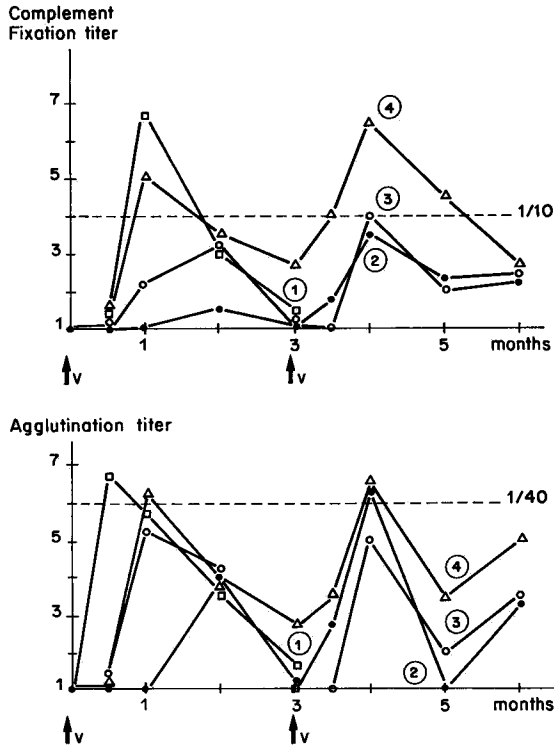


FIG. 2. — Titers of *Brucella* antibodies in agglutination and complement fixation tests in experiment A. Geometric means of 4 guinea pigs randomly taken per group; 3 indicates 50 p. 100 reaction at dilution 1/5. Group 10⁹ not given, but identical to group 10⁸. V: vaccination. 1: 10⁶ VSC; 2: 10⁶ VC; 3: 10⁷ VC; 4: 10⁸ VC.

Évolution des titres des agglutinines et des anticorps fixant le complément au cours de l'expérience A.

Moyennes géométriques des titres de 4 cobayes par lot; le lot 10⁹, identique au lot 10⁸, n'est pas représenté. Le chiffre 3 représente la réaction 50 p. 100 à la dilution 1/5. V: vaccinations.

In complement fixation, the responses were less than 1/10 for doses 10⁶ and 10⁷ by the conjunctival route even after the second vaccination. With the higher doses the response was more pronounced especially after the second vaccination. Vaccination with 10⁶ bacteria by the subcutaneous route induced an earlier response in agglutination and a higher response in complement fixation than the vaccinations by the conjunctival route.

According to the object of this experiment then, the serological responses to vaccination by the conjunctival route limit the choice of doses to the two lowest (10⁶ and 10⁷ bacteria). With these doses good protection was obtained only when re-vaccination was performed. The efficacy of this procedure was compared with the standard vaccination in the following experiments.

TABLE 2
Experiment B
Expérience B

Vaccination			Protection	Intensity of infection	
Dose	Number	Route (Voie)		Spleen (Rate)	Lymph nodes (Ganglions)
7×10^9	1	SC	11/27 (40.7 %)	2.0	0.6
10^6	2	C	10/26 (38.5 %)	3.2	1.1
10^6	2	SC	12/16 (75 %)	0.7	0.4
$7 \times 10^9 + 10^6$	2	SC + C	18/27 (67 %)	1.1	0.5
Control			0/20 (0)	5.4	2.8

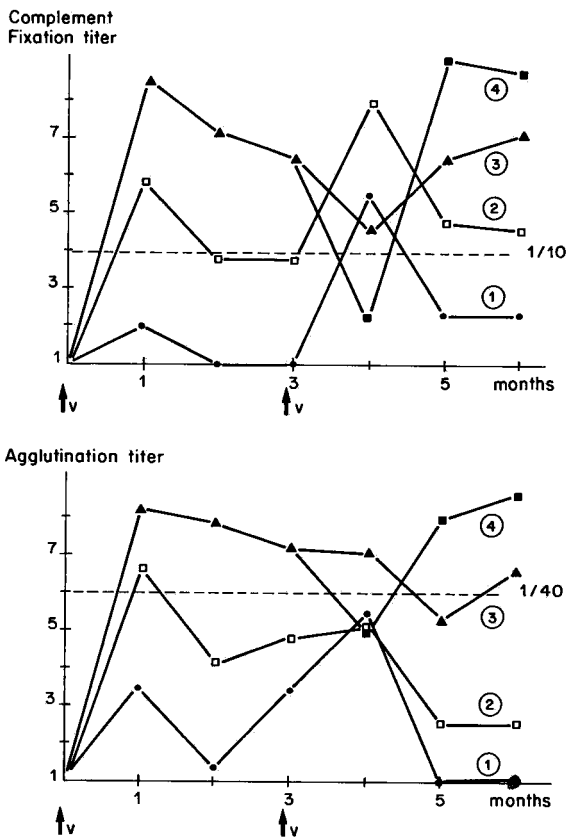


FIG. 3. — Titers of antibodies in experiment B
Geometric means of 6 guinea pigs per group
1 : 10^6 VC ; 2 : 10^6 VSC ; 3 : 7×10^9 VSC ; 4 : 7×10^9 VSC + 10^6 VC
Évolution des titres des anticorps dans l'expérience B
Moyenne des titres de 6 cobayes par lot

2. — *Experiment B : Efficacy of two vaccinations
by the conjunctival route with 10⁶ bacteria*

Vaccination by the conjunctival route with two successive doses of 10⁶ bacteria conferred protection similar to that obtained with the standard vaccination, *i.e.* a single dose of 7×10^9 bacteria by the subcutaneous route (table 2).

Re-vaccination by the conjunctival route, with a dose of 10⁶ bacteria increased the immunity conferred by the standard vaccination from 40.7 to 67 p. 100 protection which is at the limit of significance at the 10 p. 100 level ($\chi_c^2 = 2.68$ with $\chi_{0.1}^2 = 2.71$).

Two vaccinations with 10⁶ bacteria by the subcutaneous route gave the best protection (75 p. 100), better than that obtained with the same two vaccinations by the conjunctival route (38.5 p. 100, $\chi_c^2 = 3.93$ with $\chi_{0.05}^2 = 3.84$) or by the standard vaccination (40.7 p. 100, $\chi^2 = 3.46$).

The serological response is shown in figure 3. A dose of 7×10^9 bacteria by the subcutaneous route gave high titers which were increased after the re-vaccination with 10⁶ bacteria by the conjunctival route. The dose of 10⁶ bacteria by the subcutaneous route induced an average response which was increased after the re-vaccination especially in complement fixing antibodies. The dose of 10⁶ bacteria by the conjunctival route, caused only low titers even after re-vaccination, as observed in Experiment A.

It seems, then, that the dose of 10⁶ bacteria given two times by the conjunctival route is not sufficient to confer an immunity as high as, for example, that obtained by the same two doses administered by the subcutaneous route.

TABLE 3

*Experiment C**Expérience C*

Vaccination			Protection	Intensity of infection	
Dose	Number	Route (Voie)		Spleen (Rate)	Lymph nodes (Ganglions)
7 × 10 ⁹	1	SC	12/30 (40 %)	3.0	0.8
10 ⁷	2	C	14/30 (46.7 %)	2.1	0.4
7 × 10 ⁹ + 10 ⁷	2	SC + C	14/29 (48.3 %)	2.5	0.8
7 × 10 ⁹ + 10 ¹⁰ ⁽¹⁾	2	SC + SC	13/28 (46.4 %)	2.5	0.8
Control			0/4 (0)	6.1	2.5

⁽¹⁾ Second vaccination with vaccine 45/20 (killed rough *Brucella* in adjuvant).
(Deuxième vaccination avec le vaccin 45/20 (*Brucella* rough tuées avec adjuvant)).

3. — *Experiment C : Efficacy of two vaccinations
by the conjunctival route with 10^6 bacteria*

The protection obtained in this experiment (table 3) is of the same order in all groups as that obtained with the standard vaccination (40 p. 100). Thus, two vaccinations with 10^7 bacteria by the conjunctival route gave 46.7 p. 100 protection, standard vaccination followed by conjunctival re-vaccination gave 48.3 p. 100 protection, and standard vaccination followed by a second vaccination with vaccine 45/20 gave 46.4 p. 100 protection.

Although only small differences were observed, we can see that the protection and the level of infection of the groups vaccinated twice by the conjunctival route were better than that obtained with the standard vaccination in this experiment, whereas the reverse was true in Experiment B with doses of 10^6 bacteria.

DISCUSSION

Experiments A, B and C followed the same protocol with the exception of those groups in Experiment A receiving only a single vaccination. In order to increase the number of animals per group and to reduce the variation inevitable in this type of experiment, we combined the data from similar treatment groups in the three experiments. Groups in Experiment A receiving two doses of 10^8 or 10^9 bacteria were pooled, and groups in Experiment B and C receiving a booster dose of 10^6 or 10^7 after the standard vaccination were pooled. Table 4 gives the summarized results.

TABLE 4

Pooled results of experiments A, B and C

Résultats globaux des expériences A, B et C

Vaccination			Protection	Intensity of spleen infection (Infection de la rate)
Dose	Number	Route (Voie)		
10^6	2	C	16/34 (47 %)	2.69
10^7	2	C	18/36 (50 %)	1.93
10^8 or 10^9	2	C	12/20 (60 %)	1.02
10^6	2	SC	12/16 (75 %)	0.77
7×10^9	1	SC	23/57 (40 %)	2.57
$7 \times 10^9 + 10^8$ or 10^7	2	SC + C	32/56 (57 %)	1.86
Control			0/45 (0)	3.61

The standard vaccination gave the lowest rate of protection (40 p. 100). A second vaccination by the conjunctival route with a low dose (10^6 or 10^7 bacteria) 3 months after the standard vaccination increased the protection rate (57 p. 100) although

this difference was not significant at the 5 p. 100 level ($\chi_c^2 = 2.55$). Vaccination by administration of two low doses at 3 month interval by the subcutaneous route gave the best results (75 p. 100 protection), significantly better than the standard vaccination ($\chi_c^2 = 4.70$) but not better than the standard vaccination followed by the conjunctival vaccination ($\chi_c^2 = 1.00$). Vaccination with two small doses instilled in the conjunctiva at 3 month interval gave protection which, within the limit of our experiments, seemed to be dependent upon dose. However, the intermediate dose of 10^7 bacteria, with 50 p. 100 protection, was not either significantly superior to the standard vaccination ($\chi_c^2 = 0.48$) nor significantly inferior to vaccination with two doses of 10^6 bacteria by the subcutaneous route ($\chi_c^2 = 1.90$).

Despite the pooling of data the number of experimental animals per group is still insufficient to demonstrate significant differences in protection, if they exist, attributable to route of administration or dose, within groups receiving two vaccinations at intervals of 3 months. At the level of protection observed (from 50 to 75 p. 100) it would be necessary, in fact, to have more than 100 animals per group to demonstrate such differences (MAINLAND, HERRERA and SUTCLIFFE, 1956).

The serological responses to the different methods of vaccination were as expected: a significant response after standard vaccination followed by a distinct secondary response after the re-vaccination with a low dose by the conjunctival route; moderate response after vaccination with the low dose by the subcutaneous route and secondary response after the re-vaccination; weak or no response after vaccination by the conjunctival route with doses of 10^6 or 10^7 , even after re-vaccination.

We do not have available sufficient data to discuss the mechanism of immunization obtained under our conditions. At the most, one can say that the administration of a very small dose of living vaccine by the conjunctival route limits the diffusion of the antigen, in effect its multiplication or its survival, to a limited lymphatic area, the regional lymph nodes. The cells stimulated under these conditions are those which are, without doubt, particularly suitable for inducing a response of the cellular type. Furthermore, because the synthesis of antibody was minimum, there wasn't reverse antigenic competition between the receptor sites of the competent lymphocytes and those of immunoglobulins, so that the cellular response was very much greater than one would think possible with the very small quantity of antigen actually utilized.

The application of this method of vaccination to cattle requires a careful study of the suitable vaccine dose: a dose too weak, equivalent for example to 10^6 bacteria in guinea pigs, would give insufficient immunity; a dose too high, equivalent to 10^8 or 10^9 in the guinea pigs, would give an undesirable serological response. We will examine this problem in the next report.

These studies are certainly not the first where the efficacy of vaccination with a living vaccine against brucellosis have been studied utilizing doses and routes of inoculation other than the normal. STABLEFORTH (1959) reviewing the studies on cattle employing the intracaudal and intradermal routes with doses 1/5 and 1/25, respectively, of the standard dose, concluded that the serological responses and immunity were of the same order as those following subcutaneous vaccination with these same doses. Reduced doses, administered by the subcutaneous route have been studied by ALTON (1969 *a, b*) in guinea pigs, and by ALTON *et al.* (1972) in goats with the living vaccine Rev. 1 against infection with *B. melitensis*. The protection conferred

by the reduced doses was of the same order as that given by the normal dose and the serological responses of both the guinea pigs and goats were lower with the reduced dose. On the other hand, SELIVANOV (1956) used a normal dose of living vaccine B 19 administered by the conjunctival route first in guinea pigs, then in sheep (SELIVANOV and KOSHUKOV, 1959). He concluded that with the same vaccine dose (10^8 bacteria) the serological responses were similar, whether the route of inoculation was conjunctival or subcutaneous, but the protection against experimental infection was better after conjunctival vaccination. However, the numbers of animals per group in his experiment do not really permit this conclusion. In an experiment carried out in an infected flock, SELIVANOV *et al.* (1959) compared the efficacy of vaccination with 4×10^8 bacteria of B 19 by the conjunctival and intradermal routes. The number of abortions were, respectively, 8.6 p. 100 (of 704 sheep) and 11.5 p. 100 (of 440 sheep).

Thus the different methods of use of living vaccines revealed in the literature utilized either high vaccine doses administered by various routes, or lower doses by the subcutaneous route. None, to our knowledge, until now, have combined a low dose of vaccine with administration by the conjunctival route. This association in the guinea pig does not cause a significant serological response and therefore permits re-vaccination. The immunity thus obtained is equal or superior to that of the standard vaccination method.

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SUMMARY

Guinea pigs were vaccinated with different doses of strain *Brucella abortus* B 19, subcutaneously (SC) or by the conjunctival route (C), once or a second time three months later. Agglutination and complement fixation titers were determined on sera of guinea pigs taken at random at intervals from each vaccine group. Protection afforded by the vaccination was determined by intramuscular injection of the challenge strain, *B. abortus* 544 (5×10^8 bacteria) three months after the last vaccination. Forty days later the guinea pigs were killed and *Brucella* looked for and counted in the spleen and lymph nodes.

After conjunctival vaccination with 10^6 or 10^7 bacteria, serological responses were weak and transient even after the re-vaccination, and were lower than after subcutaneous vaccination with the 10^6 dose, and much lower than after the standard vaccination dose of 7×10^8 given subcutaneously.

Protection, expressed as percentage of guinea pigs free of infection at autopsy (table 4) was 40 p. 100 in the standard vaccination group (SC, 7×10^8) and 57 p. 100 in groups receiving successively 7×10^8 (SC) and 10^6 or 10^7 (C). Two successive conjunctival vaccinations with the 10^6 to 10^8 doses gave 47 to 60 p. 100 protection; two SC vaccinations with the 10^6 dose gave 75 p. 100 protection. All the control guinea pigs were heavily infected. The differences in protection rate among the groups vaccinated twice were not significant. Since vaccination through the conjunctiva with two successive instillations of 10^7 bacteria induced only a weak antibody response and protection equivalent to that of standard subcutaneous vaccination, the method appears worthy of application in cattle.

Key-words : *Antibodies, B19, Brucella abortus, guinea-pig, vaccination (conjunctival route).*

RÉFÉRENCES BIBLIOGRAPHIQUES

- ALTON G. G., 1969 *a*. Vaccination of guinea pigs with Rev. I. *Brucella melitensis* vaccine. The response to very large and very small doses. *Res. veter. Sci.*, **10**, 326-328.
- ALTON G. G., 1969 *b*. A trial in guinea pigs of four methods of vaccination against *Brucella melitensis* infection. *Res. veter. Sci.*, **10**, 329-331.
- ALTON G. G., JONES L. M., GARCIA-CARRILLO C., TRENCHI A., 1972. *Brucella melitensis* Rev. I and *Brucella abortus* 45/20 vaccines in goats: Immunity. *Amer. J. veter. Res.*, **33**, 1747-1751.
- MAINLAND D., HERRERA L., SUTCLIFFE M. I., 1956. *Statistical tables for use with binomial samples — contingency tests, confidence limits, and sample size estimates*. New York University College of Medicine, éditeur (table X, p. 82).
- PHILIPPON A., PLOMMET M., RENOUX G., GESTIN J., FENSTERBANK R., 1971. Brucellose bovine expérimentale. VII. Influence sur l'évolution de l'infection d'une faible concentration d'oxytétracycline dans l'organisme au moment de l'inoculation. *Ann. Rech. vétér.*, **2**, 147-157.
- RENOUX G., PLOMMET M., PHILIPPON A., 1971. Microréactions d'agglutination et de fixation du complément pour le diagnostic des Brucelloses. *Ann. Rech. vétér.*, **2**, 263-269.
- SELIVANOV A. V., 1956. (Immunisation de cobayes par le vaccin antibrucellique vivant NIIEG par voie conjonctivale). *J. Microbiol. Épidémiol., Immunobiol.*, **27**, 86-91 (en russe).
- SELIVANOV A. V., KOSHUKOV S. D., 1959. Vaccinal reactions and immunity in sheep inoculated with strain 19 conjunctivally or subcutaneously. *Veterinariya, Moscow*, **36**, 29-30 (in *Veter. Bull.*, 1959, **29**, 672).
- SELIVANOV A. V., KRAVETS A. T., KOSILOV I. A., 1959. Field trials of the conjunctival method of immunizing sheep against brucellosis with strain 19. *Sborn. Rabot Sibirsk. nauchnoissled vet. Inst.*, **8**, 71-74 (in *Veter. Bull.*, 1960, **30**, 553).
- STABLEFORTH A. W., GALLOWAY I. A., 1959. *Infectious diseases of animals. Diseases due to Bacteria*, vol. 1. Butterworths Scientific publications, London, 72-91.
- THORNTON D. H., MUSKETT J. C., 1972. The use of laboratory animals in the potency test of *Brucella abortus* S. 19 vaccine. *J. comp. Pathol.*, **82**, 201-208.
- VERGER J. M., 1971. Comparaison des doses infectieuses 50 p. 100 de *Brucella melitensis* inoculée par les voies conjonctivale, intragastrique et intrapéritonéale à la souris. *Ann. Rech. vétér.*, **2**, 185-196.