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Contagious bovine pleuropneumonia : Serologic response of cattle after single and double vaccination with T/1 culture vaccine *

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SUMMARY

The serologic response of cattle in 2 vaccination trials are reported. Data are presented on the duration of positive serologic reactions as measured by the rapid and overnight complement fixation, indirect hemagglutination, and serum agglutination slide tests, and the presence of circulating antigen as determined by the agar gel diffusion test.

There was an anamnestic response when cattle were revaccinated after a 3-month interval, but no detectable response when revaccination was after 1 month.

There were substantial differences between the serologic reactions of cattle vaccinated in the tail and those vaccinated subcutaneously behind the shoulder.

The merit of the agar gel diffusion test for detection of circulating antigen in infected and vaccinated cattle is discussed.

Although the T/1 culture vaccine has been used in East Africa for many years to protect cattle against contagious bovine pleuropneumonia (CBPP), there are no published data on the serologic response of cattle following vaccination. Thus, a positive serologic test may be due to previous CBPP vaccination or an active infection with *Mycoplasma mycoides* var. *mycoides*, the causative organism of CBPP.

To assess the extent and duration of serologic response to cattle after a single and double vaccination, at intervals of 1 and 3 months, 4 serologic tests of varying degrees of sensitivity were used. Two of these tests are currently used in the field to detect CBPP : the serum agglutination slide test (SAST) and the rapid complement fixation test (CFT) of Campbell and Turner as modified by Huddart (1963). The 2 other tests best suited for laboratory use are the CFT, employing an overnight fixation period as described by KABAT and MAYER (1961), and the indirect hemagglutination test (IHAT) using red blood cells coated with a polysaccharide from *M. mycoides* based on the method of COTTEW (1960). The intervals between vaccinations were chosen to follow field practice. In mass vaccination campaigns and in field control measures in the face of a CBPP outbreak, cattle are usually revaccinated 1 or 3 months after the original vaccination.

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* Voir page 118. L'analyse en Français de ce travail.

MATERIALS AND METHODS

Cattle.

Experimental cattle were East African Zebu or crosses between them and breeds of European origin, 1 to 2 years old, weighing 150-300 kg.

CBPP vaccine.

T/1 culture vaccine was used throughout the experiment. Its production and the determination of number of organisms per dose were as described by BROWN *et al.* (1965). Cattle were vaccinated in the tail or behind the shoulder with 0.5 ml containing 5×10^7 to 5×10^8 organisms.

Serological Methods.

The SAST was done as described by GOURLAY (1964) and the rapid CFT used was the Turner and Campbell method as modified by GOURLAY (1965) (*). The overnight CFT was based on the method of KABAT and MEYER (1961). Complement fixing antigen was prepared by the method of CAMPBELL and TURNER (1953) and diluted to give 2 antigen units per aliquot as determined by chessboard titrations. All sera for the CFT were diluted 1 : 10 with veronal buffered saline containing 0.1 p. 100 gelatin and heated at 56 °C for 30 minutes before use. In the test, 0.4 ml each of serum and antigen and 0.5 ml of guinea pig complement (C′) containing 5-50 p. 100 units of C′ were used, with overnight fixation at 5 °C. In addition, 0.1 ml of normal unheated bovine serum was used to enhance the fixation of C′ as described by KNIGHT and COWAN (1961). Each 100 ml of this serum was absorbed by incubation with 1 ml of packed sheep erythrocytes for 15 minutes at 37 °C and after separation of the sheep cells the serum (1 ml/tube) was stored at — 20 °C. When used at a dilution of 1 : 100 the titer of a known CBPP antiserum was increased 4 to 8 fold. The endpoint was accepted as the highest dilution of serum showing 50 p. 100 or less hemolysis when visually compared to a known standard.

The IHAT was done following the method described by COTTEW (1960), but using microtiter Perspex plates with U-shaped cups. Sheep erythrocytes were sensitized with 100 µg/ml of Galactan F extracted from *M. mycoides* Gladysdale strain as described by BUTTERY and HUDSON (1967). One ml of a 1 : 10 saline dilution of test serum was absorbed by incubation with 0.3 ml packed sheep erythrocytes at 37 °C for 30 minutes. Controls included normal serum with sensitized erythrocytes, and each test serum (at a dilution of 1 : 10) with normal sheep erythrocytes. All dilutions were made in 0.05 M phosphate buffered saline at pH 7.3 containing 0.1 p. 100 gelatin. The endpoint of the assay was defined as the last dilution of serum that gave a definite agglutination pattern.

The agar gel diffusion test (AGT) was done in petri dishes containing 1 p. 100 agar and 0.15 p. 100 sodium azide in 0.16 *M*, pH 7.4, Tris (hydroxymethyl aminomethane) buffer. Wells were cut in the agar, with a center well (9 mm in diameter) circled by 4 wells (7 mm in diameter) ; the distance between edges of the central and peripheral wells was 7 mm. Hyperimmune pig antisera against *M. mycoides* was used in the center well when testing for antigen (SHIFRINE and STONE, 1967).

(*) The Huddart (1963) CF field test is a modification of the Turner and Campbell test using Perspex cups and smaller volumes, whereas the GOURLAY (1965) modification is done in test tubes.

RESULTS

In the first experiment, 14 cattle were vaccinated, 7 in the tail and 7 subcutaneously behind the shoulder. The cattle were revaccinated by the same routes one month later, and none had adverse reactions. Results of the serologic response of these cattle are presented (Table 1). Before vaccination, all sera were negative in the rapid CFT and SAST. However, 3 sera were positive in the overnight CFT and 13 were positive in the IHAT.

After vaccination, sera from 2 of the 7 cattle inoculated in the tail were positive in the rapid CFT while 4 were positive in the SAST. By the 17th day, post-vaccination sera from all cattle were negative in both tests. Of the 7 cattle vaccinated behind the shoulder, 3 were positive in the rapid CFT and 2 were positive in the SAST ; only 1 remained positive in both tests up to the date of revaccination. At the time of revaccination, the sera of all 14 cattle were positive in the IHAT and 12 were positive in the CFT.

The revaccinated cattle remained negative in the rapid CFT, but 1 (750) had a transient positive reaction immediately after vaccination. The titers in the overnight CFT and IHAT remained the same or were reduced after revaccination.

In the second experiment, 7 cattle were vaccinated in the tail and revaccinated by the same route 3 months later. A record of the serologic response of these cattle is presented (Table 2).

All sera before vaccination were negative in the rapid and overnight CFT and SAST but positive in the IHAT. After vaccination, 4 sera were positive in the rapid CFT and 6 were positive in the SAST. All sera were negative in the rapid CFT by the 28th day post-vaccination and negative in the SAST 86 days post-vaccination. All sera were positive in both the overnight CFT and IHAT 3 to 8 days post-vaccination and were still positive at the time of revaccination 3 months later. After revaccination, sera from 3 cattle became positive in the rapid CFT, whereas 2 of 7 sera became positive in the SAST ; all sera had an increase in titer in both the overnight CFT and IHAT. The experiment was terminated 2 months after revaccination at which time sera from all cattle were negative in the rapid CFT and SAST. Although the overnight CFT and IHAT were still positive, the antibody titers were diminishing.

In both vaccination trials, sera from only 7 cattle had circulating antigens as measured by the AGT; all of these reactions were transient (Table 1, 2).

DISCUSSION

The 2 vaccination experiments here reported were designed to answer 3 questions : a) the duration of the serologic response, after an initial vaccination and after a repeat vaccination at monthly or 3-month intervals, as measured by 4 serologic methods ; b) whether there is an anamnestic response when cattle are revaccinated at monthly or 3-month intervals ; c) whether there is a difference between the serologic response of cattle inoculated in the tail or subcutaneously behind the shoulder.

The serologic response of the cattle to vaccination was transient as measured by the 2 least sensitive methods, which are also widely used for detection of CBPP. A larger number of sera were positive in the SAST (12/21) than in the rapid CFT (9/21). All cattle in both experiments were negative in the rapid CFT by the 30th day after primary vaccination, whereas some SAST reactions persisted to the 86th day. This information should be considered when assessing results of the rapid CFT and SAST in testing for CBPP infection. Neither the overnight CFT nor the IHAT could be used for testing for infection within 3 months after vaccination as sera from most cattle were still positive at this time. The IHAT is a poor method for detecting CBPP as most animals are positive in the test before vaccination.

There was no measurable anamnestic response when cattle were revaccinated after 1 month. However, in cattle revaccinated after a 3-month interval, there was an increase in antibody titer as measured by the rapid CFT, overnight CFT, IHAT and SAST. Sera from all cattle were positive in the overnight CFT and IHAT 68 days after revaccination ; 2 sera from cattle that were positive in the rapid CFT became negative 21 days post revaccination, one serum remained positive for 42 days. The 2 cattle that became positive to the SAST, after revaccination, gave negative results 21 days post revaccination.

There were substantial differences in the serologic response in cattle inoculated in the tail or subcutaneously behind the shoulder. None of the cattle had lesions at the site of inoculation. Vaccination behind the shoulder is preferred in field work because of the ease of inoculation and certainty of delivery and retention of inoculum. A large field trial to ascertain the safety of such vaccination procedure is needed. HUDSON (1965) compared tail-tip and shoulder vaccination using KH3J egg vaccine and found a higher proportion of sera from tail-vaccinated cattle gave positive SAST and rapid CFT reactions than those from the shoulder-vaccinated cattle, but the evidence was not conclusive.

The value of a precipitin test for detection of *M. mycoides* antigen and antibody was described by TURNER (1962). He showed that the most useful application of his test (interface precipitin test) was for detection of antigen. GOURLAY (1965) reported that the AGT for antigen and antibody detected 100 p. 100 of cattle with acute

TABLE 1

Serological response of cattle vaccinated twice at a one month interval

N° of animal (vaccinated in tail)	Prebleed	Days after vaccination							Days after revaccination			
		3	5	7	10	13 titer[1]	17	24	1	8	15	29
797	a. 0	0	0	0	0	0	0	0	0*	0*	0*	0
	b. 0	0	0	40	40	80	40	0	0	0	0	0
	c. 20	40	80	320	320	160	160	80	40	40	40	40
	d. -	-	-	+	+	-	-	-	-	-	-	-
755	a. 0	0	0	0	0	0	0	0	0	0	0	0
	b. 0	0	0	0	0	0	40	40	40	40	40	NT[3]
	c. 20	20	40	80	320	NT	80	80	80	80	80	80
	d. -	-	-	-	-	-	-	-	-	-	-	-
746	a. 0	0	0	20	40	20	0	0	0	0	0	0
	b. 0	0	40	40	40	40	40	40	40	40	0	0
	c. 20	80	160	640	1280	640	640	320	160	80	0	NT
	d. -	-	-	+	+	+	-	-	-	-	-	-
733	a. 0	0	0	0	0	0	0	0	0	0	0	0
	b. 0	40	40	40	80	80	40	40	40	40	40	40
	c. 20	40	80	160	160	80	80	80	80	NT	80	80
	d. -	-	-	-	-	-	-	-	-	-	-	-
751	a. 0	0	0	0	0	0	0	0	0	0	0	0
	b. 0	0	0	0	0	40	40	40	40	40	40	0
	c. 40	80	80	80	NT	NT	160	160	160	40	20	20
	d. -	-	-	+	+	-	-	-	-	-	-	-
734	a. 0	0	0	0	0	0	0	0	0	0	0	0
	b. 0	0	0	0	40	40	40	40	40	40	40	40
	c. 20	80	160	NT	320	320	160	160	80	80	NT	NT
	d. -	-	-	-	-	-	-	-	-	-	-	-
748	a. 0	0	0	0	0	0	0	0	0	0	0	0
	b. 80	80	160	80	160	160	80	80	80	80	80	80
	c. 20	80	160	NT	320	80	160	160	80	NT	40	40
	d. -	-	-	+	+	-	-	-	-	-	-	-

TABLE I

(following)

N° of animal (vaccinated behind shoulder)	Prebleed	Days after vaccination										
		3	5	7	10	13 titer[1]	17	24	1	8	15	29
802	a. 0	0	0	0	0	0	0	0*	0*	0*	0*	0
	b. 0	40	80	80	80	80	80	160	80	80	0	0
	c. 20	40	20	80	NT	NT	80	80	40	40	20	80
	d. -	-	-	-	-	-	-	-	-	-	-	-
744	a. 0	0	0	0	0	0	0	0	0	0	0	0
	b. 0	0	0	0	0	80	40	40	NT	40	40	40
	c. 10	10	80	160	320	160	160	160	40	40	40	80
	d. -	-	-	-	-	-	-	-	-	-	-	-
738	a. 0	0	0	0	0	0	0	0	0	0	0	0
	b. 0	0	0	0	40	80	40	40	40	0	0	0
	c. 10	10	80	80	80	1280	80	40	40	40	40	80
	d. -	-	-	-	-	-	-	-	-	-	-	-
750	a. 0	0	0	0	40	40	40	40	40	20	0	0
	b. 40	40	80	80	160	160	160	160	160	160	160	160
	c. 40	80	160	160	NT	80	320	320	160	160	40	40
	d. -	-	-	-	±	+	+	+	+	-	-	-
741	a. 0	0	0	0	0	20	0	0	0	0	0	0
	b. 0	40	40	40	40	80	80	80	80	80	0	0
	c. 0	40	80	80	NT	NT	160	160	80	80	20	20
	d. -	-	-	-	-	-	-	-	-	-	-	-
747	a. 0	0	0	0	0	0	0	0	0	0	0	0
	b. 40	40	40	40	80	80	40	80	80	40	0	0
	c. 10	80	40	80	NT	160	80	40	80	NT	20	NT
	d. -	-	-	-	-	-	-	-	-	-	-	-
739	a. 0	0	0	0	40	40	0	0	0	0	0	0
	b. 0	0	0	0	40	40	80	0	0	0	0	0
	c. 10	20	40	NT	320	NT	1280	320	80	NT	40	40
	d. -	-	-	+	+	+	-	-	-	-	-	-

1. The titer is expressed as the reciprocal of the serum dilution at which the end point occurred

2. a. complement fixation test (60 mins. fixation)
   b. complement fixation test (overnight fixation)
   c. indirect hemagglutination test
   d. slide agglutination serum test; results are expressed as -, + or ± doubtful

3. Not Tested

* Positive for (circulating) antigen in ACT

TABLE 2

Serological response of cattle vaccinated twice at a three month interval

N° of animal	Prebleed	Days after vaccination														Days after revaccination							
		3	8	14	21	28	36 titer[1]	43	49	57	64	71	78	86	92	7	14	21	28 titer[1]	35	42	56	70
870	a. 0	0	0	20	40*	0	0	0	0	0	0	0	0	0	0	80	80	0	0	0	0	0	0
	b. 0	40	40	40	80	40	40	40	40	40	40	20	20	20	20	160	80	NT[3]	20	20	40	40	20
	c. 40	20	1280	320	80	40	40	80	40	40	160	80	40	20	40	80	160	NT	80	160	80	80	80
	d. -	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
875	a. 0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0*	0*	0	0	0	0	0	0
	b. 0	0	40	40	40	40	80	80	80	80	80	40	40	20	20	160	NT	NT	40	40	40	40	40
	c. 80	160	1280	320	160	160	160	160	160	160	320	160	160	40	40	160	320	160	160	160	80	160	160
	d. -	-	-	+	-	-	+	+	+	+	-	-	+	-	-	-	-	-	-	-	-	-	-
926	a. 0	0	0	10	0	0	0	0*	0	0	0	0	0	0		0	0	0	0	0	0	0	0
	b. 0	40	20	40	40	40	40	40	40	40	20	0	20	20	20	80	80	NT	20	20	20	0	0
	c. NT	80	1280	320	320*	NT	160	80	160	80	80	160	80	40	80	160	160	160	80	160	80	80	80
	d. -	-	-	-	-	-	-	+	-	-	-	-	-	-		-	-	-	-	-	-	-	-
929	a. 0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	b. 0	40	20	80	80	80	0	0	40	40	20	10	0	20	NT	40	40	NT	20	40	10	10	10
	c. 20	NT	160	160	320*	20	20	20	20	20	40	40	20	20	20	20	40	20	40	40	20	20	40
	d. -	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-
958	a. 0	0	0	0	0	0	0	0	0	0	0	0		0	0	160	160*	0*	0	0	0	0	0
	b. 0	0	80	0	40	40	80	80	80	40	40	40	40	10	10	160	80	NT	20	40	20	20	20
	c. 40	80	160	40	40	40	40	40	40	80	80	80	40	20	40	320	320	160	80	80	40	40	40
	d. -	-	-	-	-	-	-	+	-	-	-	-	+	+	-	+	+	-	-	-	-	-	-
974	a. 0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	160	80	80	80	80	40	0	0
	b. 0	0	80	160	160	80	40	40	40	20	20	10	0	20	20	80	80	NT	20	40	20	20	20
	c. 20	40	40	320	320	160	80	40	80	40	80	40	40	20	40	40	80	80	40	80	40	40	40
	d. -	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
980	a. 0	0	0	0	0	0	0	0	0	0	0	0	0*	0*	0	0	0	0	0	0	0	0	0
	b. 0	0	20	20	40	40	40	40	40	40	20	20	20	20	20	80	40	NT	20	10	10	10	10
	c. 20	40	320	320	160	80	160	80	80	40	80	80	80	20	40	80	80	80	80	160	80	40	40
	d. -	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-

1. The titer is expressed as the reciprocal of the serum dilution at which the end point occurred.

2. a. complement fixation test (60 mins. fixation)
   b. complement fixation (overnight fixation)
   c. indirect hemagglutination test
   d. slide agglutination serum test

3. Not Tested

* Positive for (circulating) antigen in the AGT

CBPP but only 21 p. 100 with chronic CBPP. In a subsequent study, SHIFRINE and GOURLAY (1967), using the AGT for antigen only, could detect about 80 p. 100 of both acute and chronic CBPP cases. In this study, vaccinated cattle have only a transient phase during which circulating antigen is detectable by the AGT. The AGT thus becomes a useful test for checking cattle for CBPP soon after vaccination when the other tests may still give positive results because of vaccination, and for differentiating between infected and vaccinated cattle. Because of its simplicity and economy, the value of this test for field work was reported previously (SHIFRINE and GOURLAY, 1967 ; SHIFRINE, 1967).

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RÉSUMÉ

Péripneumonie contagieuse bovine :
réponse sérologique des bovins à la vaccination
à simple et double dose par le vaccin T_1 de culture

La réponse sérologique du bétail dans deux essais de vaccination est étudiée ; des résultats sont rapportés, sur le titre et la durée anticorps vaccinaux qu'on a recherchés par les tests suivants : séroagglutination sur lame, fixation rapide et lente du complément, hémagglutination conditionnée.

La recherche de l'antigène circulant a été faite par précipitodiffusion en milieu gélifié.

On n'observe de phénomène de rappel que lorsque les 2 vaccinations successives sont faites à 3 mois d'intervalle et non à 1 mois d'intervalle.

La différence de sites d'injection (extrémité de la queue et zone post-scapulaire) entraîne une différence de réponse sérologique

L'utilité du test de diffusion en gélose pour la recherche de l'antigène circulant est discutée.

RESUMEN

Perineumonia contagiosa bovina : respuesta serológica
de los bovinos para la vacunación, con sola y doble dosis
de la vacuna T_1 de cultivo

Se estudia la respuesta serológica del ganado durante dos ensayos de vacunación. Se dan los resultados sobre el título y la duración de los anticuerpos vaccinales buscados mediante las pruebas siguientes : seroaglutinación en lámina, fijación rápida y lenta del complemento, hemaglutinación condicionada. La búsqueda del antigeno circulante fué realizada por precipitación-difusión en medio gelificado. Solo se observa un fenómeno de reacción cuando se hacen las 2 vacunas sucesivas a los 3 meses y no al mes de intervalo.

La diferencia de localizaciones de la inyección (extremidad del rabo y zona post-escapularia) tiene por consecuencia una diferencia de la respuesta serológica.

Se discute la utilidad de la prueba de difusión en gelosa para la búsqueda del antigeno circulante.

Retour au menu

RÉFÉRENCES

BROWN (R. D.), GOURLAY (R. N.) & MacLEOD (A. K.). — **The production of TI broth culture contagious bovine pleuropneumonia vaccine.** *Bull. epiz. Dis. Afr.* (1965) **13** : 149-155.

BUTTERY (S. H.) & HUDSON (J. R.). — **Personal communication,** 1967.

CAMPBELL (A. D.) & TURNER (A. W.). — **Studies on contagious bovine pleuropneumonia. IV. An improved complement fixation test.** *Aust. Vet. J.* (1953) **29** : 154-163.

COTTEW (G. S.). — **Indirect haemagglutination and haemagglutination inhibition with *Mycoplasma mycoïdes*** *Aust. Vet. J.* (1960) **36** : 54-56.

GOURLAY (R. N.). — **Antigenicity of *Mycoplasma mycoïdes*. I. Examination of body fluids from cases of contagious bovine pleuropneumonia.** *Res. Vet. Sci.* (1964) **5** : 473-482.

GOURLAY (R. N.). — **Comparison between some diagnostic tests for contagious bovine pleuropneumonia.** *J. Comp. Path.* (1965) **75**: 97-109.

HUDDART (J. E.). — **A field modification of the complement fixation for contagious bovine pleuropneumonia.** Animal Health Monograph N° 6, Food and Agriculture Organization, Rome (1963), 65 pp.

HUDSON (J. R.). — **Contagious bovine pleuropneumonia : the immunizing value of the attenuated strain KH3J.** *Aust. Vet. J.* (1965) **41** : 43-49.

KABAT (E. A.) & MAYER (M. M.). — **Experimental Immunochemistry.** C. C. Thomas Pub., Springfield, Ill., U. S. A. (1961).

KNIGHT (G. J.) & COWAN (K. M.). — **Studies on allegedly non-complement-fixing immune systems. I. A heat labile serum factor requirement for a bovine antibody complement fixing system.** *J. Immunol.* (1961) **86**: 354-360.

SHIFRINE (M.). — **A rapid gel diffusion precipit in test for contagious bovine pleuropneumonia.** *Bull. Wildlife Disease Assoc.* (1967) **3** : 36

SHIFRINE (M.) & GOURLAY (R. N.). — **Evaluation of diagnostic tests for contagious bovine pleuropneumonia.** *Bull. epiz. Dis. Afr.* (1967) **15** : 7-10.

SHIFRINE (M.) & STONE (S. S.). — **A serological comparison of virulent and avirulent strains of *Mycoplasma mycoïdes*.** In preparation.

TURNER (A. W.). — **Detection of *Mycoplasma mycoïdes* antigen and antibody by means of precipit in tests, as aids to diagnosis of bovine contagious pleuropneumonia.** *Aust. Vet. J.* (1962) **38** : 335-337.

PÉRIPNEUMONIE CONTAGIEUSE BOVINE : RÉPONSE SÉROLOGIQUE DES BOVINS A LA VACCINATION A SIMPLE ET DOUBLE DOSE PAR LE VACCIN T_1 DE CULTURE

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(Analyse)

Bien que le vaccin de culture de la souche T_1 soit employé depuis longtemps en Afrique Orientale pour lutter contre la péripneumonie, il n'existe encore aucune donnée précise sur la réponse sérologique post-vaccinale du bétail.

Une réaction sérologique positive pourrait être due aussi bien à une vaccination antérieure qu'à une infection en évolution.

Aussi était-il intéressant d'évaluer l'intensité et la durée de cette réponse, chez des bovins vaccinés en un seul temps et en deux temps (vaccination double) à 1 et 3 mois d'intervalle.

Quatre méthodes sérologiques, de sensibilité différente, furent utilisées ; les deux premières, séroagglutination sur lame et test de fixation rapide du complément sont couramment employées sur le terrain, les deux autres sont habituellement réservées au laboratoire : fixation du complément lente (une nuit) et test d'hémagglutination indirecte.

Les intervalles de 1 ou de 3 mois entre les deux vaccinations sont ceux que l'on utilise dans les campagnes de vaccination et dans les mesures d'extinction des foyers de péripneumonie.

MATÉRIEL ET MÉTHODES

Les animaux étaient des zébus Est-africains ou des métis zébu - races européennes, âgés de 1 à 2 ans et pesant 150 à 300 kg.

Le vaccin T_1 était injecté soit à la queue, soit derrière l'épaule à la dose de 0,5 ml (5.10^7 à 5.10^8 germes).

Les méthodes sérologiques citées répondaient aux normes suivantes :

1. séroagglutination sur lame, selon GOURLAY (1964).

2. fixation rapide du complément, effectuée selon le protocole de HUDDARD (1963), mais en tubes.

3. fixation lente du complément, selon KABAT et MAYER (1961) avec 2 unités d'antigène et chauffage de tous les sérums à 56 °C pendant 30 minutes après dilution au 1/10 dans un tampon au véronal contenant 0,1 p. 100 de gélatine. La fixation du complément (5 UH_{50}) dure une nuit à 5 °C et chaque tube reçoit en outre 0,1 ml de sérum frais négatif de bovin de façon à accroître le titre de fixation (KNIGHT et COWAN, 1961).

4. hémagglutination indirecte selon une méthode quantitative, inspirée de celle de COTTEW (1960), en plaques de plexiglas et avec des hématies sensibilisées par 100 ug/ml de galactane. Les dilutions sériques sont faites en tampon phosphate de pH 7,3 et à 0,1 p. 100 de gélatine.

5. précipito-diffusion en gel dans le milieu suivant :

gélose	1 g
azide de sodium	0,15 g
tampon Tris, 0,16 M, à pH 7,4	100 ml

Avant la vaccination, tous étaient négatifs à la S. L. (Séroagglutination sur lame) et à la F. R. (*) (fixation rapide) ; deux étaient positifs à la F. L. (*) (fixation lente) et à l'H. I. (hémaglutination indirecte).

RÉSULTATS

1. Dans la première expérience, 14 animaux furent vaccinés, 7 à la queue, 7 derrière l'épaule ; tous furent revaccinés un mois plus tard.

Pour les vaccinés à la queue, 2 deviennent positifs en F. R. et 4 en S. L. Au 17e jour, tous sont devenus négatifs à ces deux tests.

Pour les vaccinés à l'épaule, 3 deviennent positifs en F. R. et 2 en S. L. ; un seulement reste positif à ces deux tests jusqu'au jour de la revaccination.

A ce moment, les 14 bovins sont positifs en H. I. et 12 en F. L.

La deuxième intervention laisse les animaux négatifs en F. R. à l'exception d'un qui accuse une réaction positive fugace immédiatement après l'intervention. Les titres en H. I. et F. L. restent les mêmes ou se réduisent.

2. Dans la seconde expérience, 7 animaux sont vaccinés à la queue et revaccinés au même endroit 3 mois après.

Au départ, tous les sérums sont négatifs en F. R., F. L. et S. L., mais positifs en H. I. Après vaccination, 4 deviennent positifs en F. R. et 6 en S. L. avec ces deux méthodes, tous sont redevenus négatifs respectivement 28 jours et 86 jours après la vaccination.

Entre 3 et 8 jours après celle-ci, ils étaient tous positifs en F. L. et en H. I. et le sont restés jusqu'à la seconde intervention 3 mois plus tard.

(*) S. L. = S. A. S. T. du texte en langue anglaise.
F. R. = C. F. T. du texte en langue anglaise.
F. L. = overnight C. F. T. du texte en langue anglaise.
H. I. = I. H. A. T. du texte en langue anglaise.

Après cette dernière, tous les sérums eurent leur titre accru en F. L. et H. I., mais 3 et 2 animaux devinrent positifs en F. R. et S. L.

L'exp érience se termina deux mois après; les 7 bovins étaient alors négatifs en F. R. et S. L. et positifs aux tests de F. L. et H. I. (avec cependant un titre diminué).

Au cours des deux expériences, 7 animaux seulement eurent, de façon transitoire, de l'antigène circulant dans leur sérum.

DISCUSSION

1. Il est à retenir que les bovins sont négatifs 30 jours après une primovaccination en F. R. et 86 jours après en S. L.

Ni la F. L. ni la H. I. ne sont à utiliser pour dépister la maladie sur du bétail vacciné au cours des 3 mois précédents.

En outre, la plupart des animaux sont positifs en H. I. avant même qu'ils soient vaccinés.

2. Il n'y a pas de réponse anamnestique décelable lorsque la seconde vaccination a lieu au bout de 30 jours ; par contre, à trois mois d'intervalle, celle-ci provoque une augmentation du titre des anticorps.

On a pu observer un animal qui restait positif en F. R. jusqu'à 42 jours après ; il a fallu 21 jours pour que deux autres redeviennent négatifs en S. L.

3. Il y a des différences significatives entre la réponse des animaux vaccinés à la queue et celle des animaux vaccinés derrière l'épaule. Ce dernier site d'inoculation est préféré en pratique à cause de sa facilité d'accès.

4. On ne peut déceler l'antigène circulant dans le sérum des vaccinés que très temporairement, aussi le test de précipitation en milieu gélifié est-il très utile pour faire le diagnostic de péripneumonie évolutive peu de temps après une vaccination, alors que les autres tests sont tous positifs.