Bull. Org. mond. Santé }
Bull. Wld Hlth Org. } 1962, **26**, 409-419

Immunization with Viable *Brucella* Organisms *

Results of a Safety Test in Humans

WESLEY W. SPINK, M.D., JAMES W. HALL III, M.D.
JOANNE FINSTAD & EDMUND MALLET

In many parts of the world contact with infected livestock may involve a serious risk of the spread of human brucellosis. Partial control of bovine brucellosis has been achieved by slaughter of infected herds and immunization of cattle with Brucella abortus *strain 19 living vaccine. However, in areas where such measures are unpractical there remains a need for protection of humans. This study compares the safety of two living* Brucella *vaccine preparations in human volunteers.*

Some 32 healthy male volunteers with no evidence of past exposure to brucellosis were divided into two comparable groups; one group received 19-BA vaccine derived from Br. abortus *and the other received Rev 1 vaccine derived from* Br. melitensis. *Detailed studies over a six-month period of the clinical effects, bacteraemia,* Brucella *agglutinin response and dermal hypersensitivity revealed striking differences between the two groups. Two of the 16 men in the 19-BA group developed acute brucellosis, and another had a positive blood culture. In the Rev 1 group, 11 of 16 developed acute brucellosis, and brucellae were recovered from 12.*

All 32 men developed Brucella *agglutinins, the Rev 1 group having higher titres. Dermal hypersensitivity occurred in all of the Rev. 1 group but in only nine of the 19-BA group.*

Tetracycline treatment in all the Rev 1 group and in the two brucellosis cases in the 19-BA group resulted in complete recovery.

The authors conclude from this study that neither the Rev 1 vaccine nor the 19-BA vaccine inoculated subcutaneously is sufficiently safe in the dosage used to warrant being used for vaccination of humans for prophylactic purposes.

Human brucellosis results from direct or indirect contact with several species of infected animals, notably, cattle, sheep, goats and swine. Therefore eradication of the disease in animals will result in the elimination of human illness. Efforts to control animal brucellosis have included the testing and slaughtering of diseased livestock and the vaccination of cattle. Such control programmes have been highly successful in cattle, with a corresponding decrease in the incidence of human brucellosis due to *Brucella abortus*. But the major challenge of animal and human brucellosis involves infection due to *Brucella melitensis*, with sheep and goats acting as the reservoir of the disease. Attempts to vaccinate sheep and goats against *Br. melitensis* are being evaluated, but there will be considerable delay before a proper vaccine can be selected and applied on a world-wide scale. In the meantime, an extensive *Brucella* immunization programme in human beings has been under way in the USSR, and as a result there has been reported a marked decrease in infections due to *Br. melitensis* among livestock workers coming in contact with sheep and goats (Vershilova, 1961). The vaccine is composed of living organisms derived from *Br. abortus* strain 19, which was originally introduced in the USA for immunizing cattle.

* From the FAO/WHO Brucellosis Centre, Department of Medicine, University of Minnesota Medical School, Minneapolis, Minn., USA.

The use of viable *Brucella* organisms for human immunization was considered at length in 1957 by the Joint FAO/WHO Expert Committee on Brucellosis (1958). On the basis of the available evidence the Committee could not recommend any vaccine for routine use because it regarded " the procedure as being still in the experimental stage ". In view of the reported success in the USSR with *Br. abortus* 19 the status of human vaccination was reviewed by a Scientific Group on *Brucella* Trials in Man convened by the World Health Organization in 1959. The group recommended that a safety trial with two vaccines should be carried out under rigidly controlled conditions. One product to be evaluated was the USSR *Br. abortus* vaccine designated 19-BA (Vershilova, 1961). The second vaccine, Rev 1, was developed by Elberg and his associates at the University of California from a strain of *Br. melitensis* with attenuated virulence (Herzberg & Elberg, 1955; Elberg & Faunce, 1957; Elberg, 1959).[1]

The group further recommended that in a vaccine trial a minimum of two groups of 10 individuals each should be given a subcutaneous injection of one of the vaccines in a dose of 2.5×10^8 organisms. It was suggested that the selected persons should be males, 21-40 years of age, living in a non-endemic area of brucellosis. Arrangements were made to carry out this safety test in the Minnesota State Prison. The details of the investigation were found to be acceptable to a Medical School Research Committee of the University of Minnesota, and to the WHO Group on Vaccine Trials. The two vaccines were administered to a total of 32 men, 16 in each group. Clinical, immunological, serological and bacteriological observations were carried out for a period of six months after the vaccine had been administered. The purpose of this report is to summarize the results.

MATERIALS AND METHODS

Adult male inmates of the Minnesota State Prison were informed in detail of the purposes and methods of the study, and 105 volunteers between the ages of 20 and 50 years were tested intradermally with *Brucella* antigen. Examinations at 24 and 48 hours showed 14.3% of the group with positive reactions. From the non-reactors 32 men were selected as potential candidates and subjected to a medical history, general physical examination, and determination of serum antibody titres to *Brucella*. Final selection was based on general good health, negative skin tests, the absence of *Brucella* agglutinins, and a length of internment exceeding the duration of the study. All subjects of necessity received the same diet and were exposed to similar environmental conditions within the prison. The 32 men were divided into two groups by assigning every other name in alphabetical order to one or the other group, and on 23 May 1960 each member of group 1 (subsequently referred to as the 19-BA group) was injected subcutaneously with the strain 19-BA *Br. abortus* vaccine, and group 2 (subsequently referred to as the Rev 1 group) with the strain Rev 1 *Br. melitensis* vaccine.

The inmates selected were generally of good muscular development, but they were moderately overweight owing to the restricted exercise imposed by the winter months. No significant physical abnormalities were present. The average age of the 19-BA group was 41.3 years (range 31-50 years) and of the Rev 1 group 38.9 years (range 30-50 years).

Follow-up examinations, agglutination tests, and blood cultures were carried out on all subjects at weekly intervals for the first month, then at three and six months following vaccination. Oral temperatures were determined twice daily. In addition the men were seen each day during the first two weeks, and later re-examined immediately whenever symptoms occurred, at which time appropriate diagnostic and therapeutic measures were carried out as described below. Intradermal skin tests were repeated at three- and six-month intervals after vaccination.

Vaccines

The 19-BA vaccine was supplied by Professor P. A. Vershilova of the USSR Academy of Medical Sciences, Moscow, and was prepared from a subculture of *Br. abortus* strain 19, which—unlike typical *Br. abortus* isolates—possesses attenuated virulence and does not require added 10% CO_2 for growth. The results of the injection of the living organisms into guinea-pigs and into large numbers of humans in the USSR indicated that this preparation was safe (Vershilova, 1961). Two-day agar cultures suspended in 10% saccharose and 1% gelatin in distilled water were lyophilized and supplied for use in sterile vials. The contents were reconstituted with isotonic saline to yield a population of approximately 2.5×10^8 viable *Brucella* organisms per ml. One millilitre was immediately injected subcutaneously into the deltoid region.

[1] See also the article by Elberg & Faunce on page 421 of this issue.

The Rev 1 vaccine was supplied by Dr Sanford S. Elberg of the University of California. The derivation of this attenuated strain from virulent *Br. melitensis* has been described elsewhere (Herzberg & Elberg, 1955; Elberg & Faunce, 1957). Extensive investigations with this living vaccine in mice, guinea-pigs, goats and monkeys indicated that only minor systemic reactions occurred, and significant protection against challenge with more virulent *Brucella* strains was obtained (Herzberg & Elberg, 1955; Elberg, 1959).[1] Protection of goats against *Br. melitensis* infection under conditions of natural infection in Malta has been reported by Alton (1959, 1961). Freshly lyophilized cultures prepared from 3% lactose in Zobell's solution (Heckly et al., 1960) were assayed and provided by Dr Elberg so that 2.5×10^8 living organisms were present in 0.5 ml. After reconstitution with isotonic saline 0.5 ml was immediately injected subcutaneously into the deltoid region. Both vaccines had been approved for trial by the WHO Scientific Group on *Brucella* Vaccine Trials in Man.

Skin test antigen

Polyvalent antigen composed of concentrated extracts of *Br. melitensis*, *abortus* and *suis* was prepared and standardized according to nitrogen content by Dr M. Ruiz Castañeda of Mexico City, Mexico, and was diluted 1 : 100 in 0.5% phenolized isotonic saline for intradermal injection (Castañeda & Cardenas, 1941). The preparation was first tested in 100 randomly selected patients at the University of Minnesota Hospitals and found to be slightly more sensitive in eliciting positive responses than Brucellergen, a commercially available nucleoprotein fraction of Huddleson et al. (1943). A 0.1-ml quantity was injected intradermally with a 25-gauge (0.50 mm) needle into the forearm, and the results were interpreted at 24 and 48 hours. Criteria of grading were as follows: 0=erythema and induration less than 1 cm; 1+ = redness and swelling of 1 cm; 2+=2 cm of redness and swelling; 3+=marked erythema and induration, sometimes with lymphadenopathy. A definite positive reaction was recorded if 1+ or greater reaction occurred at 48 hours.

Agglutination tests

The antigen used was *Br. abortus*, strain 1119, prepared by the Agricultural Research Service, US Department of Agriculture. The method used has been described previously (Spink, 1956; p. 196). Under these conditions a titre of 1 : 160 or 1 : 320 represents 160 or 320 International Antibody Units per ml respectively (Joint FAO/WHO Expert Committee on Brucellosis, 1958). "Blocking antibodies" were tested for after centrifugation at 2500 r.p.m. for 10 minutes (Spink, 1956; p. 199).

Blood cultures

A 10-ml quantity of freshly drawn venous blood was added to standard "double media" bottles containing citrated Albimi broth and citrated Albimi agar along one vertical surface. When incubated at 37°C, colonies were usually noted on the agar surface after between 5 and 14 days. In all instances, 1-2 ml of broth culture were transferred to large Albimi agar slants at intervals up to 30 days. Identification of recovered organisms was established by Gram stain, growth on agar plates containing basic fuchsin and thionin dyes, and slide agglutination with rabbit anti-*Brucella* sera. The organisms from the urine culture were isolated after culture on Albimi agar and similarly identified.

Observations following vaccination are presented as direct comparisons between the two groups with regard to (1) signs and symptoms of illness, (2) blood cultures, (3) appearance of *Brucella* agglutinins and (4) intradermal *Brucella* tests.

CLINICAL OBSERVATIONS

Local reaction to vaccines

Local reactions of different degrees of intensity occurred, as summarized in Table 1. The injection itself did not elicit pain beyond that expected from skin puncture. Local effects varied from no visible reaction and absent subjective sensation to marked erythema and induration with moderate swelling of the arm, lymphadenopathy, and itching or pain. These manifestations were definitely more prominent with the 19-BA vaccine, 13 of 16 recipients showing significant reactions. Twelve individuals in the Rev 1 group showed local effects, but these were less prominent than in the 19-BA group. One man had fever, lymphangitis, supraclavicular lymphadenopathy and a diffuse soreness in the arm. In all instances the local erythema, soreness, and swelling subsided in 3-4 days.

Systemic reactions.

Striking differences in systemic reactions between men of the two groups were expressed. The manifes-

[1] See also the article by Elberg & Faunce on page 421 of this issue.

TABLE 1

COMPARISON OF LOCAL REACTIONS WITH TWO *BRUCELLA* VACCINES IN 32 VOLUNTEERS

Vaccine 19-BA		Vaccine Rev 1	
Patient No.	Reaction	Patient No.	Reaction
1	Very slight	1	None
2	Slight	2	Sore arm
3	7 cm swelling	3	None
4	None	4	None
5	3 cm swelling	5	None
6	4 cm swelling; redness	6	Soreness and redness
7	3.5 cm swelling	7	Slight swelling
8	3.5 cm swelling, soreness	8	Large, tender supraclavicular node
9	4 cm swelling	9	Slight
10	3.5 cm swelling	10	Moderate swelling, fever
11	3.5 cm swelling	11	Slight soreness
12	3.5 cm swelling	12	Slight soreness
13	4.5 cm swelling	13	Sore arm
14	4 cm swelling	14	Slight swelling
15	4 cm swelling	15	Slight soreness
16	3 cm swelling	16	Slight soreness

tations and bacteriological results are outlined in Table 2. Among the 16 men vaccinated with 19-BA, two (Nos. 11 and 13) demonstrated evidence of acute brucellosis. Patient No. 13 had a temperature of 103°F (39.4°C), chills, malaise, and nausea beginning on the second day and necessitating hospitalization. Symptoms gradually subsided in four days without specific treatment. Patient No. 11 had sweats and malaise, and the spleen was palpable 5 cm below the left costal margin. Symptoms subsided in three days following tetracycline therapy. In neither of these men were brucellae recovered from the blood-stream. The only positive culture occurred on the 22nd day in an asymptomatic, afebrile individual (No. 15). One additional patient (No. 6) became quite ill with tonsillitis and then a rectal abscess. Coagulase-negative staphylococci were cultured from the blood-stream. This illness was not diagnosed as brucellosis, and the patient improved on hospitalization and antibiotics. Two blood cultures from Patient No. 1, who felt entirely well, showed coagulase-negative staphylococci, the significance of which is not clear.

In striking contrast, 11 (69%) of the 16 men who received Rev 1 vaccine became ill with acute brucellosis, four requiring hospitalization (Table 2). The onset of illness began between the third and 23rd days, the majority occurring in the second week. These men were sicker than the two affected in the 19-BA group, with more protracted symptoms and inability to return to work of from 3 to 17 days, or an average of 6.4 days of disability. The symptoms and signs were characteristic of acute brucellosis and included the following : fever of 99°-104°F (37.2°-40°C), chills, sweats, headache, body aches, anorexia, nausea, epigastric pains, depression, sore throat, dysuria, weight loss, lymphadenopathy, and splenomegaly.

In all those of the Rev 1 group with brucellosis, except one (No. 11), brucellae were cultured from the blood, or, as in No. 14, from the urine. In two others (Nos. 6 and 13) brucellae were recovered from the blood, but symptoms did not warrant the diagnosis of acute brucellosis. The total number of men who received Rev 1 vaccine and had positive cultures was 12 of 16, and eight of these had demons-

TABLE 2

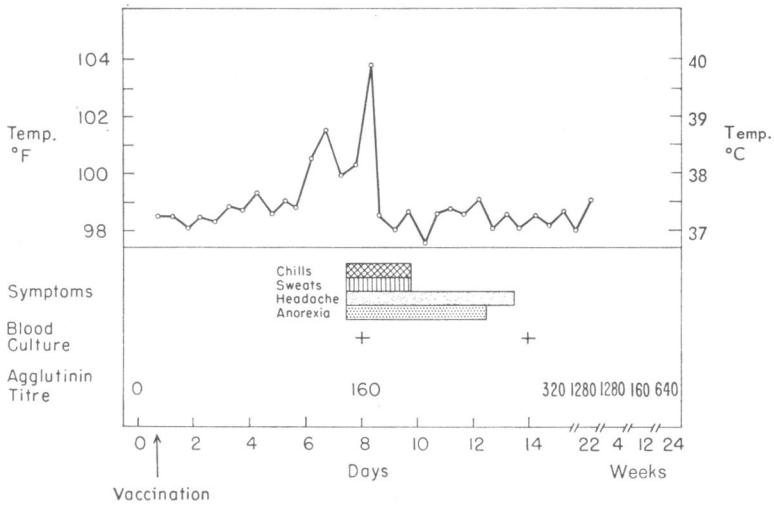
CLINICAL OBSERVATIONS AND BACTERIOLOGICAL DATA FOLLOWING VACCINATION WITH LIVING *BRUCELLA*

Vaccine 19-BA						Vaccine Rev 1					
Patient No.	Systemic reaction		Hospitalized	Blood cultures		Patient No.	Systemic reaction		Hospitalized	Blood cultures	
	Day			Day	Organism		Day			Day	Organism
1		None		8	+ *Staph.*	1 [a]	7	Fever, chills, sweats, headache, anorexia		8	+ *Brucella*
				22	+ *Staph.*					9	+ *Brucella*
										15	+ *Brucella*
2		None			0						
						2 [a]	3	Fever, chills, body aches		8	+ *Brucella*
3	2	Mild diarrhoea			0					15	+ *Brucella*
										22	+ *Brucella*
4	3	Mild conjunctivitis			0	3 [a]	8	Chills, malaise, aches		8	+ *Brucella*
										9	+ *Brucella*
5		None			0						
						4 [a]	14	Fever, headache, backache		15	+ *Brucella*
6	10	Tonsillitis, fever	Yes							17	+ *Brucella*
	19	Rectal abscess, fever		8	+ *Staph.*	5 [a]	16	Fever, chills, headache, anorexia	Yes	15	+ *Brucella*
										17	+ *Staph.*
7	2	Aches in legs			0						
	11	Headache				6	9	Sweats, sore throat, headache, muscle stiffness, nausea		15	+ *Brucella*
										22	+ *Brucella*
8	10	Slight headache			0	7		None			0
9		None			0	8 [a]	9	Fever, chills, headache, anorexia	Yes	8	+ *Brucella*
										9	+ *Brucella*
10		None			0						
						9 [a]	10	Fever, chills, headache, body aches	Yes	8	+ *Brucella*
11 [a]	2	Malaise								15	+ *Brucella*
	17	Sweats, splenomegaly	Yes		0	10 [a]	11	Fever, joint pains, abdominal pains		8	+ *Brucella*
										15	+ *Brucella*
12		None			0						
						11 [a]	10	Headache, body aches, sore throat			0
13 [a]	2	Fever, chills, malaise, nausea	Yes		0	12 [a]	17	Fever, headache, body aches		15	+ *Brucella*
14		None			0	13	8	" Cold ", headache, body aches	Yes	15	+ *Brucella*
15	4	Slight headache		22	+ *Brucella*	14 [a]	23	Fever, chills, headache, conjunctivitis, spleen tender, pyelonephritis		24	+ *Brucella* (urine)
16		None			0	15		None			0
						16	15	Slight fever, headache, body aches			0

[a] Patients with acute brucellosis.

FIG. 1

SEQUELAE FOLLOWING VACCINATION WITH LIVING *BRUCELLA MELITENSIS* IN PATIENT NO. 1 OF REV 1 VACCINE GROUP



trable bacteraemia on more than one occasion, yielding a total of 22 *Brucella* isolates. Table 3 summarizes these findings.

The course of brucellosis in Patient No. 1 of the Rev 1 group, a 37-year-old Negro male, is illustrative of the typical sequelae after inoculation with the Rev 1 vaccine (Fig. 1). No antibiotic treatment was given during the acute illness, thus the " natural " course of infection was not altered for this period. Subcutaneous injection with the living vaccine was well tolerated and he felt normal until six days later, when fever began. At eight days severe chills, flushing, and sweats suddenly developed, followed by malaise, anorexia, and throbbing frontal headache. He was hospitalized immediately and all symptoms persisted for two days, after which the temperature became normal. He continued to have generalized malaise and headache for a total of six days. He then returned to work and, except for slight weight loss, felt well. Nine days after recovery tetracycline therapy was begun (this was the time when treatment was instituted for all of the Rev 1 group). Blood drawn on day 8, coincident with the onset of symptoms, on day 9, and on day 15, shortly after recovery, grew *Br. melitensis*. *Brucella* agglutinins, which were absent prior to vaccination, became demonstrable in a titre of 1: 160 on day 8, 1: 320 on day 15, and 1: 1280 by the third and fourth weeks. The titre then decreased to 1: 160 at three months, but was 1: 640 at six months. On follow-up examinations at three and six months the patient was well, and blood cultures were sterile.

Several of the patients with brucellosis in the Rev 1 group received treatment with tetracycline within 48 hours of the onset of illness. When it became apparent that the majority of these men were manifesting acute brucellosis, it was elected on the 25th day following vaccination to treat all of them with tetracycline, 0.5 g four times daily (or its equivalent [1]) for 21 days. Improvement occurred within three to four days after therapy was started. Since treatment with tetracycline was begun at a time when new cases were still appearing it is possible that additional instances of acute brucellosis

TABLE 3

CASES OF ACUTE BRUCELLOSIS AND NUMBER OF *BRUCELLA* ISOLATES IN 32 VOLUNTEERS RECEIVING TWO STRAINS OF LIVING *BRUCELLA* VACCINE

	Vaccine 19-BA (16 men)	Vaccine Rev 1 (16 men)
No. of acute cases of brucellosis	2 (12 %)	11 (69 %)
No. with positive *Brucella* cultures	1 (6 %)	12 (75 %)

[1] In the form of Cosa-Tetracyn (Pfizer) 250 mg four times a day.

were prevented in members of the Rev 1 group. All signs and symptoms of illness in members of both groups—except Patient No. 14 in the Rev 1 group—had subsided by one month after vaccination, and at three and six months all men were in good health.

Patient No. 14, Rev 1, presented the special problem of protracted renal infection. Following vaccination he was well until the 23rd day, when fever, chills, and severe malaise appeared suddenly, followed by throbbing frontal headaches and body aches. He appeared acutely ill with a temperature of 102° F (38.9°C), conjunctivitis, and tenderness in the splenic area. Frequent and burning urination began the following day, associated with frank pyuria, and brucellae were cultured from the urine. A diagnosis of acute *Brucella* pyelonephritis was made, hospitalization effected, and treatment initiated with 1 g daily of tetracycline. Fever and generalized symptoms gradually decreased over five days; however, urinary frequency and burning persisted, so a daily dose of 1 g of Gantrisin (sulfisoxazole) and 2 g of streptomycin was added for two weeks. Three weeks after the onset of illness all therapy was stopped. He continued to feel well, except for mild urinary frequency, and he returned to work. From then on he had normal urine analyses and urine cultures remained sterile.

Agglutinin response

All members of both groups had no *Brucella* agglutinins prior to vaccination. Table 4 summarizes the serial changes in titres determined at one, two, three, and four weeks, and then at three and six months following vaccination. Blood specimens were obtained prior to repeated skin testing. The pattern of antibody rise and fall was quite uniform for each individual, seldom deviating more than one or two tubes in the weekly determinations. These data are diagrammatically presented in Fig. 2. Moderate increases in titre appeared in the first week and were similar for both groups; however, beginning in the second week, the agglutinins of the Rev 1 group began to rise significantly above those of the 19-BA group. By the fourth week all members of Rev 1 had titres of 1: 320 or greater. A titre of 1: 320 is often taken as the usual significant level in the diagnosis of acute brucellosis (Spink, 1956; p. 102) but less than half of the 19-BA group at

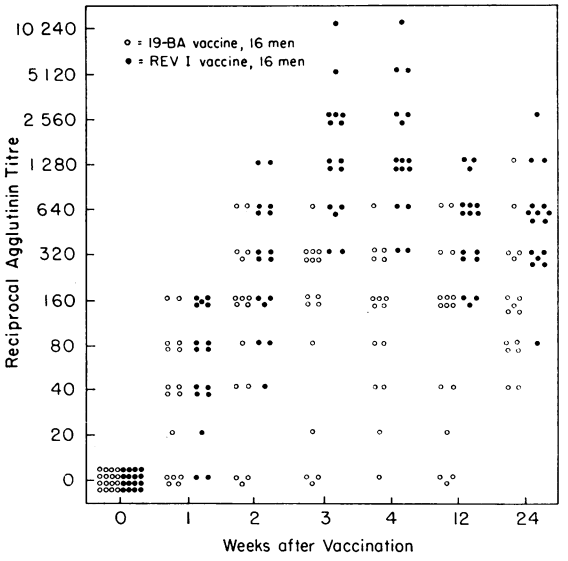
TABLE 4

RECIPROCAL SERUM AGGLUTINATION TITRES FOLLOWING INOCULATION WITH TWO *BRUCELLA* VACCINES

Vaccine 19-BA							Vaccine Rev 1						
Patient No.	Week						Patient No.	Week					
	1	2	3	4	12	24		1	2	3	4	12	24
1	160	320	320	160	320	320	1	160	320	1 280	1 280	160	640
2	0	80	80	80	320	320	2	0	80	1 280	320	160	80
3	0	160	320	320	160	160	3	80	2 280	2 560	1 280	320	320
4	160	160	160	320	160	80	4	40	320	1 280	1 280	640	640
5	80	640	320	160	160	80	5	80	160	2 560	1 280	640	320
6	40	40	160	160	40	160	6	40	160	640	640	320	640
7	80	160	160	80	20	40	7	20	80	320	320	320	640
8	40	0	0	40	0	40	8	160	640	10 240	10 240	1 280	640
9	0	0	0	0	0	160	9	0	640	5 120	2 560	640	640
10	0	0	0	20	0	160	10	160	1 280	2 560	2 560	640	320
11	80	320	640	640	640	1 280	11	160	640	2 560	5 120	640	1 280
12	0	640	320	320	160	80	12	80	640	640	1 280	640	640
13	80	320	320	160	160	320	13	80	320	2 560	5 120	1 280	1 280
14	20	40	20	40	40	80	14	160	320	640	2 560	1 280	2 560
15	40	160	160	160	160	160	15	40	0	320	640	320	320
16	40	160	320	320	640	640	16	40	160	1 280	1 280	160	320

FIG. 2

AGGLUTININ RESPONSE FOLLOWING INOCULATION WITH LIVING *BRUCELLA* VACCINES (19-BA AND REV 1) IN 32 INDIVIDUALS



three months showed these values and several failed to show any titre at all, although six months after vaccination all individuals in this group had demonstrable agglutinins. The agglutinin titres of the Rev 1 members exhibited much higher individual elevations—some up to 10 240, which was the last dilution studied—and the titres remained higher for this group up to the end of the sixth month.

Skin tests

The 32 men had negative skin tests prior to vaccination, as formerly noted. Intradermal tests with *Brucella* antigen were done three and six months later, and interpreted at 24 and 48 hours. The reactions, graded 0 to 3+ as defined previously, are shown in Table 6 below. In the 19-BA group at three months, eight (50%) had become positive as judged by the final 48-hour evaluation, and at six months only nine (56%) were positive. Three non-reactors at three months (Nos. 3, 6, 16) converted to positive at six months, while two with positive tests (Nos. 4, 12) at three months reverted to negative on the second test. The pattern with Rev 1 recipients was much more uniform; 15 men (95%) having positive reactions at three months and all 16 (100%) being positive at six months. Table 5 is a summary of these results. There were no instances of significant systemic reaction to skin testing at either time.

TABLE 5

TOTAL INCIDENCE OF POSITIVE SKIN TESTS (48 HOURS) AT THREE AND SIX MONTHS AFTER VACCINATION WITH TWO STRAINS OF LIVING *BRUCELLA* IN 32 VOLUNTEERS

Time	Vaccine 19-BA (16 men)	Vaccine Rev 1 (16 men)
Three months	8 (50%)	15 (94%)
Six months	9 (56%)	16 (100%)

The comparison between the skin test reactions at 48 hours and the serum agglutinin titre at the same time for each individual is tabulated in Table 6. Blood for the agglutination test was drawn just prior to injecting the skin test antigen. The lack of direct correlation is immediately apparent. For example, at three months No. 16 of the 19-BA group showed a negative skin reaction despite a titre of 1: 640, while No. 10 of the 19-BA group had a positive intradermal reaction but there were no demonstrable agglutinins. This pattern is illustrated by the random scattering observed in Fig. 3, when the degree of delayed skin hypersensitivity reaction is plotted against the concurrent antibody titres; the lack of straight line correlation is shown. At both periods the points for the Rev 1 group are upwards and to the right, emphasizing the higher agglutinin titres and more uniform delayed skin hypersensitivity for the group as a whole compared with the 19-BA group.

FIG. 3

AGGLUTININ TITRES COMPARED WITH SKIN TEST RESULTS FOR EACH VOLUNTEER AT THREE MONTHS AND SIX MONTHS AFTER VACCINATION WITH 19-BA OR REV 1 STRAINS OF *BRUCELLA*

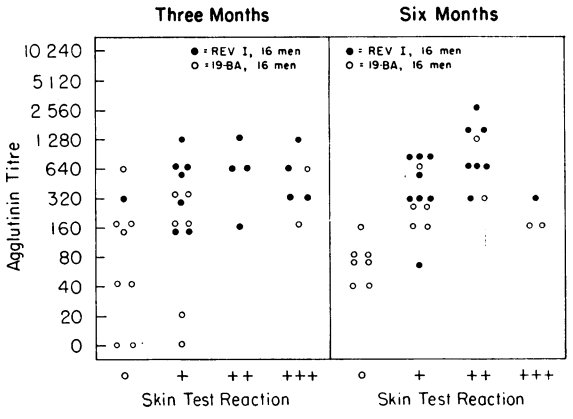


TABLE 6

48-HOUR SKIN TEST RESULTS COMPARED WITH AGGLUTINATION TITRES IN 32 VOLUNTEERS AT THREE AND SIX MONTHS

Vaccine 19-BA					Vaccine Rev 1				
Patient No.	3 months		6 months		Patient No.	3 months		6 months	
	Skin test	Aggl. titre	Skin test	Aggl. titre		Skin test	Aggl. titre	Skin test	Aggl. titre
1	+	320	+	320	1	+	160	++	640
2	+	320	+	320	2	+	160	+	80
3	0	160	+	160	3	+	320	+	320
4	+	160	0	80	4	+	640	+	640
5	0	160	0	80	5	+	640	+	320
6	0	40	+++	160	6	0	320	++	640
7	0	20	0	40	7	+++	320	++	640
8	0	0	0	40	8	+++	1 280	+	640
9	+	0	+++	160	9	+	640	+	640
10	+	0	+	160	10	+++	640	+++	320
11	+++	640	++	1 280	11	++	640	++	1 280
12	+++	160	0	80	12	++	640	+	640
13	+	160	++	320	13	++	1 280	++	1 280
14	0	40	0	80	14	+	1 280	++	2 560
15	0	160	0	160	15	+++	320	++	320
16	0	640	+	640	16	++	160	+	320

DISCUSSION

The primary purpose of this study was to determine whether either of two *Brucella* vaccines could be safely given to human beings. The results with the Rev 1 vaccine were quite decisive. The signs and symptoms of acute brucellosis appeared in 11 of the 16 volunteers, and it was necessary to hospitalize four of the men. Brucellae were recovered from the blood cultures of 11 of 16 recipients of the vaccine. In several individuals the cultures were positive two and three weeks following vaccination. One of the men (No. 14) developed *Brucella* pyelonephritis. Fortunately, all of the volunteers recovered after treatment with tetracycline. This vaccine cannot be recommended for human immunization.

Although fewer cases of acute brucellosis occurred in the group receiving the 19-BA vaccine, four of the 16 individuals had undesirable sequelae. Two of the subjects (Nos. 11 and 13) developed acute brucellosis and had to be hospitalized. A third subject (No. 6) became quite ill from tonsillitis on the tenth day after receiving the vaccine, and coagulase-negative staphylococci were cultured from the blood. Shortly thereafter he developed a rectal abscess. It is not clear whether this man's illness can be ascribed to the vaccine. Viable 19-BA organisms were recovered from the blood of a fourth man (No. 15) three weeks after receiving the vaccine, when headache was also present. These results show that the use of strain 19-BA in humans is associated with a significant degree of risk.

In evaluating strain 19-BA for human vaccination the extensive experience of the investigators in the USSR must be considered. Vershilova (1961) has reported that following the vaccination of over 3 000 000 persons the incidence of human brucellosis due to *Br. melitensis* was reduced by nearly 60% over the period 1952-58, although there was no reduction in the incidence of brucellosis in animals. According to Vershilova vaccine 19-BA is innocuous, although she states: "Some persons (8%) complained of general malaise and headache. A rise in temperature from 37.2°C to 37.6°C for 24-48 hours

was observed rarely (2%)." It is implied that more serious reactions were attributed to *Brucella* hypersensitivity occurring in individuals who either had been previously vaccinated or had latent brucellosis.

The conflicting conclusions of the present study and those of the workers in the USSR concerning the safety of vaccine 19-BA must be considered in relation to the problem of human brucellosis at hand. In areas where there is a high incidence of brucellosis due to *Br. melitensis* and little likelihood of eradicating the disease in animals in the immediate future, the risk of using a prophylactic living vaccine must be equated with its effectiveness in reducing the natural incidence of human disease. In some areas in the world the incidence of human brucellosis has been markedly reduced in a relatively short period of time by the eradication of the disease in livestock. Therefore, it would be highly undesirable in such localities to introduce the risk of a living vaccine.

Turning to some of the other features of this study, it is apparent that dermal *Brucella* hypersensitivity was more readily acquired and more prominent with the Rev 1 vaccine. All of the volunteers receiving Rev 1 developed positive skin reactions for *Brucella* antigen, while five of 16 receiving 19-BA failed to do so. The uniform acquisition of *Brucella* hypersensitivity by the Rev 1 group is probably related to a more widespread proliferation of the organisms in the tissues of the host.

All 32 volunteers revealed *Brucella* agglutinins six months after the injection of vaccine. During the entire period the agglutinin titres of those receiving Rev 1 tended to be higher than those given the 19-BA vaccine. This serological disparity also reflects the more widespread parasitization of the tissues by Rev 1. Every individual with positive blood cultures had demonstrable *Brucella* agglutinins as well as a positive *Brucella* skin test.

The behaviour of Rev 1 in human volunteers is in sharp contrast with the attenuated virulence demonstrated by Elberg and his associates in mice, guinea-pigs, goats, sheep and monkeys (Herzberg & Elberg, 1955 ; Elberg & Faunce, 1957).[1] Failure to establish disease in lower animals as compared with the results obtained in man emphasizes the desirability of careful safety tests in man with any new viable vaccine.

[1] See also the article by Elberg & Faunce on page 421 of this issue.

RÉSUMÉ

Les auteurs ont procédé à l'étude comparative de deux vaccins antibrucelliques, BA-19 et Rev 1, obtenus respectivement à partir de germes atténués de *Brucella abortus* et de *Br. melitensis*. Ces vaccins ont été injectés à 32 volontaires indemnes de brucellose dans leur passé et répartis en deux groupes de composition identique. Pendant les six mois qui ont suivi la vaccination, chaque individu a été maintenu en observation et soumis à intervalles réguliers à divers examens de laboratoire. L'hémoculture, le séro-diagnostic et l'intradermo-réaction, ainsi que les réactions tant locales que générales, ont fait apparaître des différences significatives, voire marquées, entre les 2 groupes.

Le groupe BA-19 a compté 2 cas de brucellose aiguë et 1 hémoculture positive chez un sujet asymptomatique. Ces chiffres ont été respectivement de 11 et de 12 dans le groupe Rev 1, où la maladie a revêtu par ailleurs des formes plus graves.

Les 32 sujets en expérience ont tous produit des agglutinines, mais dès la 4e semaine tous les vaccinés au Rev 1 avaient un taux d'agglutination égal ou supérieur à 1:320, valeur qui, au 3e mois, n'était atteinte que par 4 vaccinés du groupe BA-19.

Bien qu'il n'existe pas de corrélation entre les taux d'agglutination et l'apparition ou l'intensité de l'intradermo-réaction, cette dernière, plus marquée et plus précoce, était présente au 6e mois chez tous les sujets du groupe Rev 1; à cette date, elle n'a pas été retrouvée chez 7 vaccinés au BA-19.

Aucun de ces deux vaccins, et particulièrement le Rev 1, ne peut être recommandé pour l'immunisation des populations contre la brucellose, sauf dans les régions où cette affection a une fréquence globale très élevée, et où l'éradication ne peut être obtenue dans un proche avenir par abattage systématique des animaux contaminés. Dans la période 1952-58, le vaccin BA-19 utilisé en URSS a réduit de 60% la fréquence de la brucellose dans une population de 3 000 000 d'habitants. Il comporte cependant des risques qui ne permettent pas de généraliser son emploi.

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