IMMUNOLOGICAL STUDIES ON BRUCELLOSIS WITH SPECIAL REFERENCE TO THE USE OF S.19 VACCINE IN CATTLE.

Being a Thesis submitted

for the degree of D.Sc. of Edinburgh University.

by

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C O N T E N T S

11. A Comparison of the Intradermal and Subcutaneous Routes in producing Immunity to Brucellosis in Cattle.

12. Assessing the Immunising Value of Br. abortus Vaccines in Cattle.

FOREWORD

The following thesis consists of twelve papers recording the results of an investigation concerning various aspects of brucellosis. With one exception all have been published, and this paper has now also been accepted for publication.

The work described has proceeded over a period of ten years from 1943 to 1953 at the Agricultural Research Council's Field Station, Compton, Berkshire, the main object being the furtherance of knowledge pertaining to the immunisation of cattle against brucellosis. The technique of experimentation in cattle on a scale calculated to be sufficiently large to provide reliable results has been developed, particular attention being directed towards ensuring that the previous relevant history of the experimental animals was known. Special isolation precautions have been evolved to ensure that no extraneous infection could gain access to the animals during the experimental period and, to enable comparisons to be made between different immunising antigens, a standardised method of infecting the experimental cattle with brucellosis has now been established. Much of the information so far obtained, has already been utilised by the Ministry of Agriculture and Public Health authorities in this and other countries to assist in formulating policies designed

to prevent the occurrence of brucellosis in cattle and in man. It was inevitable that certain results had to be published in conjunction with one or more colleagues because this field of research was too vast for any one individual to cover alone. Accordingly, in order to maintain continuity, these four additional papers have been incorporated in the text in sequence, rather than in an appendix. In these conjoint experiments the present author has been wholly responsible for all aspects of the work directly concerning the experimental cattle and in addition has performed all the serological and many of the cultural and biological tests.

ACKNOWLEDGEMENTS

These studies have been authorised by the Agricultural Research Council and the work has proceeded under the general aegis of the Council's Brucellosis Committee.

I am especially indebted to Dr. W.S. Gordon, Director of this Field Station for his kind encouragement and guidance throughout the last decade. Without his foresight, initiative and critical appraisal of the needs of the situation, these experiments might never have been accomplished.

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Immunity in Cattle Vaccinated with Brucella Abortus Strain 19 and a Note Comparing this Strain with 45/20

by

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In a previous paper Edwards, de Ropp and McLeod (1945) described the immunological properties and infectivity of *Br. abortus*, strain 45/20, in cattle. They found that a vaccine prepared from this strain conferred considerable immunity against infection with virulent *Br. abortus*.

The present paper describes an experiment to test, in a similar way, the immunizing value of strain 19 (Ministry of Agriculture No. 1 vaccine). The vaccine tested was obtained from the Ministry's routine issue and it will be shown that it produced a high degree of immunity in cattle against test doses of virulent *Br. abortus*, which infected all of 19 unvaccinated controls. Further, after exposure to infection and following parturition, the milk of vaccinated and control animals was examined weekly, for ten weeks, for the presence of infection and, except for two animals which failed to resist infection, the milk from the vaccinated group remained free from *Br. abortus*, whereas infection was detected in the milk of all the controls.

Materials and Methods

ANIMALS AND ACCOMMODATION.—Fifty maiden heifers, reared at this station and ranging in age from 18 to 23 months, were used; 35 were Ayrshires and 15 were British Friesians. Prior to the experiment these animals were consistently negative to the agglutination test for *Br. abortus*. They were divided into five groups of ten and each group was housed in a separate building, which was bird-proof. At the entrance to each building there was a small ante-room where personnel and equipment entering or leaving could be disinfected. Each of the five units, A, B, C, D and E, had a separate cattle attendant and the animals were identified by the letter of the unit they occupied and a serial number. They were kept in the buildings throughout the course of the experiment and were subjected to the following procedures:—

VACCINATION.—On 13.8.42 the 20 heifers in units D and E were injected subcutaneously with 5·0 ml., of the Ministry of Agriculture's anti-abortion vaccine No. 1 (*Br. abortus*, strain 19). A viable count of a sample of this vaccine showed it to contain approximately 12,000 million organisms per ml.

The 20 animals in units A and B acted as unvaccinated controls while the ten animals in Unit C, which were neither vaccinated nor infected, provided a check on the efficiency of the methods of isolation.

MATING.—Using two bulls, mating was commenced on October 18th, 1942. One bull was used for the animals in units A and E, the other for those in units B and D. In unit C, five of the heifers were served by the A, E bull and five by the B, D bull. Forty-two of the 50 heifers were effectively served between October and December, 1942, and the others, with the exception of four which were infertile, by February, 1943.

EXPOSURE TO VIRULENT INFECTION.—On April 13th, 1943, when the majority of the animals were in about the fifth month of pregnancy, those in units A, B, D and E were exposed to infection. For this purpose *Br. abortus*, strain 544, described by McEwen (1940) as a fully virulent strain, was used. A 48-hour culture on bacto-tryptose agar was washed off in Ringer's fluid and adjusted in opacity to match tube 10 on Brown's scale. This suspension was diluted tenfold and one hundredfold and viable counts of both suspensions were made by three separate workers. The mean of the readings showed that Brown $\frac{\text{tube } 10}{10}$ contained about 1,500 million viable organisms per ml., and Brown $\frac{\text{tube } 10}{100}$ contained about 150 million viable organisms per ml. The infective dose was administered by placing 0·1 ml. on the surface of the conjunctiva; the animals in units A and E were given the small test dose containing about 15 million viable organisms and those in units B and D the large test dose of about 150 million viable organisms. Tests carried out in guinea-pigs by the method described by de Ropp (1945) confirmed that the strain used was fully virulent.

AGGLUTINATION TESTS.—Blood samples for agglutination tests were obtained from all the animals at frequent intervals during the course of the experiment. The tests were carried out by the technique described by Stableforth (1936) using antigen kindly supplied by the Laboratory of the Ministry of Agriculture. The end point of the titre recorded was the highest dilution of serum which produced visible agglutination.

EXAMINATION AT PARTURITION.—At parturition the following materials were obtained for examination:—

(*a*) *Blood.* Samples were taken from the jugular vein.

(*b*) *Cotyledons.* A cotyledon was detached from the foetal membranes as aseptically as possible, preferably before the membranes had been expelled from the animal, and transferred to a sterile McCartney bottle.

(*c*) *Colostrum.* The udder was washed, dried and the teats swabbed with 50 per cent. alcohol. The first three jets from each quarter were discarded. Thereafter, a McCartney bottle was filled with approximately 5 ml. from each quarter.

(*d*) *Foetal Stomach Contents.* The abdomen of the foetus was incised, the surface of the stomach wall seared with a hot spatula and a sample of the contents transferred to a McCartney bottle by means of a Pasteur pipette.

The samples from the vaccinated animals were examined culturally and biologically, whereas samples from the controls were examined biologically only if a culture of *Brucella* was not obtained.

CULTURAL TECHNIQUE.—Plates of bacto-tryptose agar containing 2½ per cent. blood were inoculated respectively with varying quantities of gravity cream from colostrum, with a 20 per cent. suspension of pulped cotyledon in Ringer's fluid and with foetal stomach contents. In no case did the inocula exceed 0·5 ml. of the colostrum and 0·1 ml. of cotyledon suspension or foetal stomach contents. The plates were incubated aerobically and in 10 per cent. CO_2 at 37° C. for four days.

BIOLOGICAL TESTS.—Five guinea-pigs were used for each sample of material from the vaccinated animals and two for each sample from the controls. The dose consisted of 1·0 ml. injected intramuscularly. One pig of the vaccinated group was killed eight days after injection and the remainder six weeks after injection.

EXAMINATION OF MILK SAMPLES.—*Post-partum* milk samples from all animals were examined weekly for ten weeks. Those from vaccinated animals were tested culturally and biologically, whereas samples from controls were examined biologically only if no culture of Brucella was obtained. The inoculum for biological tests was 1·0 ml. gravity cream injected intramuscularly.

IDENTIFICATION OF *Br. abortus*.—Films were made from suspected colonies and slide agglutination tests prepared. If the organism resembled *Br. abortus* morphologically and serologically, it was accepted as *Br. abortus*.

Throughout the experiment all samples for cultural examination were taken in triplicate and each of the three samples was examined by a different worker. Occasionally *Br. abortus* was isolated from a particular site by one worker only and in such cases a positive result was recorded.

Results

VACCINATION.—Swellings of varying degree up to 6 in. in diameter developed at the site of vaccination and some animals showed slight constitutional disturbance which passed off in 48 hours. In one animal the swelling became indurated and persisted for the duration of the experiment.

FERTILITY RATE.—With one exception (E.2), all of the 20 vaccinated animals became pregnant, while of the 30 non-vaccinated, three were infertile. There was, therefore, no evidence that the vaccine had lowered the fertility rate in the vaccinated as compared with the non-vaccinated animals. Prior to application of the infective dose the three non-vaccinated barren animals, one in unit A and two in unit B, were exchanged for three of the non-vaccinated pregnant animals in unit C.

UNIT C.—*Non-vaccinated, non-infected Controls.* The heifers in unit C were examined regularly throughout the course of the experiment by the same methods as those adopted for animals in the other groups. All remained negative to the agglutination test and at parturition all were free from infection. In the eighth month of pregnancy, one aborted from a non-specific cause; no

TABLE I

SMALL INFECTIVE DOSE—CONTROLS (UNVACCINATED)

13.4.43 small infective dose, 15 million viable organisms *Br. abortus* Strain 544, applied to the conjunctiva

Heifer No.	Agglutination titres of serum: Weeks before infection 35	33	31	28	26	23	19	14	9	7	5	2	13.4.43	Weeks after exposure to infection 2	5	8	11	14	17	20	23	26	29	32	History of pregnancy: Days from service to infection	Days from infection to parturition	Duration of pregnancy in days	Parturition	Fate of calf	At parturition: Blood serum titre	Cotyledons Cult.	Cotyledons Biol.	Colostrum Cult.	Colostrum Biol.	Foetal stomach Cult.	Foetal stomach Biol.
A1	–	–	–	–	–	–	–	–	–	–	–	–	–	1	2	4	4	7	8	8	10	8	10	..	165	100	265	PI	L	7	+	..	—	0/2	..	..
A2	–	–	–	–	–	–	–	–	–	–	–	–	–	1	–	1	–	4	8	8	8	6	6	..	156	106	262	PI	L	6	+	..	—	0/2	..	..
A3	–	–	–	–	–	–	–	–	–	–	–	–	–	1	3	3	3	2	5	8	8	6	6	..	151	116	267	PI	D	5	+	..	—	2/2	+	..
A4	–	–	–	–	–	–	–	–	–	–	–	–	1	2	3	5	7	8	9	8	9	7	9	..	146	82	228	PI	D	7	+	..	+	..	+	..
A5	–	–	–	–	–	1	–	–	–	–	–	–	–	–	3	2	4	5	7	6	9	8	9	..	141	133	274	NI	L	7	+	..	+	..	..	..
A6	–	–	–	–	–	–	–	–	–	–	–	–	–	–	2	2	5	8	11	10	10	8	11	..	152	104	256	PI	D	9	+	..	+	..	+	..
A7	–	–	–	1	–	–	–	–	–	–	–	–	–	3	3	7	7	7	7	6	6	5	7	..	156	59	215	PI	D	7	+	..	+	..	+	..
A8	–	–	–	–	–	–	–	–	–	–	–	–	–	1	3	6	8	10	10	10	10	9	12	..	152	71	223	PI	D	7	+	..	+	..	+	..
A9	–	–	–	–	–	–	–	–	–	1	–	–	–	–	3	2	7	10	9	11	11	9	12	..	112	116	228	PI	D	9	+	..	+	..	+	..
A10	–	–	–	–	–	–	–	–	–	–	–	–	Died of anthrax 6.4.43																							

SMALL INFECTIVE DOSE—VACCINATED GROUP

13.8.42 injected subcutaneously with 5·0 ml. Ministry of Agriculture No. 1 Vaccine (*Br. abortus*, Strain 19). 13.4.43 same infective dose as controls above

	Weeks after vaccination 0	2	4	7	9	12	16	21	26	28	30	33	13.4.43 35	Weeks after exposure to infection 2	5	8	11	14	17	20	23	26	29	32												
E1	–	6	5	4	4	3	3	3	2	..	4	3	3	4	5	5	4	4	4	4	4	3	2	2	143	135	278	N	L	4	—	0/5	—	0/5	..	..
Not pregnant:																																				
E2	–	3	6	6	4	4	4	2	2	..	2	2	2	3	3	3	3	3	2	2	2	2	1	1												
E3	–	8	7	6	5	4	4	3	4	..	3	3	3	4	4	4	4	4	3	3	3	3	2	3	149	127	276	N	L	4	—	0/2	—	0/5	..	..
E4	–	7	8	7	6	5	5	4	5	..	4	4	4	6	6	6	6	6	6	5	5	5	4	4	164	124	288	N	L	6	—	0/5	—	0/5	..	..
E5	–	7	6	5	6	4	2	3	3	..	2	2	2	4	4	3	3	3	4	6	4	4	3		132	141	273	NI	L	4	—	5/5	—	3/5	..	..
E6	–	6	5	6	4	3	4	2	1	..	1	1	1	2	2	2	2	2	1	1	1	1	–	–	143	141	284	N	L	1	—	0/5	—	0/5	..	..
E7	–	3	6	6	6	6	7	6	6	..	5	5	5	6	6	6	5	5	4	4	4	4	3	4	146	135	281	N	L	4	—	0/5	—	0/5	..	..
E8	–	8	7	6	5	5	5	3	3	..	3	2	2	3	3	3	3	3	3	2	3	3	2	2	156	121	277	N	L	3	—	0/5	—	0/5	..	..
E9	–	7	7	6	4	4	5	3	3	..	3	3	3	4	4	4	4	3	3	3	3	2	2	2	116	162	278	N	L	3	—	0/5	—	0/5	..	..
E10	1	8	7	6	5	3	4	3	–	..	3	3	3	4	4	6	6	5	5	5	5	5	4	4	132	150	282	N	L	5	—	0/5	—	0/5	..	..

TABLE III

LARGE INFECTIVE DOSE—CONTROLS (UNVACCINATED)

13.4.43 large infective dose, 150 million viable organisms *Br. abortus* Strain 544, applied to the conjunctiva

Heifer No.	Agglutination titres of serum: Weeks before infection 35	33	31	28	26	23	19	14	9	7	5	2	13.4.43	Weeks after exposure to infection 2	5	8	11	14	17	20	23	26	29	32	35	38	41	44	History of Pregnancy: Days from service to infection	Days from infection to parturition	Duration of pregnancy in days	Parturition	Fate of calf	At parturition: Blood serum titre	Cotyledons Cult.	Cotyledons Biol.	Colostrum Cult.	Colostrum Biol.	Foetal stomach Cult.	Foetal stomach Biol.
B1	–	–	–	–	–	–	–	–	–	–	–	–	–	3	4	3	7	9	9	8	8	..	..	..	..	..	..	..	112	91	203	PI	D	8	+	..	+	..	+	..
B2	–	–	–	–	–	–	–	–	–	–	–	–	–	3	5	6	7	9	8	6	6	..	..	..	..	..	..	..	156	73	229	PI	D	6	+	..	—	0/2	—	2/2
B3	–	–	–	–	–	–	–	–	–	–	–	–	–	2	3	7	7	9	9	7	8	..	..	..	..	..	..	..	172	71	243	PI	D	6	+	..	+	..	+	..
B4	–	–	–	–	–	–	–	–	–	–	–	–	–	3	3	7	10	11	11	9	10	..	..	..	..	..	..	..	138	73	211	PI	D	9	+	..	+	..	+	..
B5	–	–	–	–	–	–	–	–	–	–	–	–	–	3	5	9	10	9	10	9	10	..	..	..	..	..	..	..	169	61	230	PI	D	9	+	..	+	..	+	..
B6	–	–	–	–	–	–	–	–	–	–	–	–	–	3	5	7	8	9	8	7	8	..	..	..	..	..	..	..	115	77	192	PI	D	8	+	..	+	..	+	..
B7	–	–	–	–	–	–	–	–	–	–	1	1	2	3	3	6	7	6	5	3	..	..	..	..	..	..	..	..	176	87	263	PI	L	7	+	..	—	0/2	..	..
B8	–	–	–	–	1	1	–	–	–	–	–	–	–	3	5	8	9	9	9	9	9	..	..	..	..	..	..	..	143	68	211	PI	D	8	+	..	+	..	+	..
B9	–	–	–	2	–	..	–	–	–	–	–	–	–	4	7	8	9	10	8	8	8	..	..	..	..	..	..	..	172	65	237	PI	D	8	+	..	+	..	+	..
B10	–	–	–	–	–	–	–	–	–	–	–	–	–	4	5	7	7	7	7	7	7	..	..	..	..	..	..	..	177	40	217	PI	D	5	+	..	—	2/2	+	..

LARGE INFECTIVE DOSE—VACCINATED GROUP

13.8.42 injected subcutaneously with 5·0 ml. Ministry of Agriculture No. 1 vaccine (*Br. abortus*, Strain 19). 13.4.43 same infective dose as controls above

	Weeks after vaccination 0	2	4	7	9	12	16	21	26	28	30	33	13.4.43 35	Weeks after exposure to infection 2	5	8	11	14	17	20	23	26	29	32	35	68	41	44											
D1	–	7	7	6	5	2	3	2	3	..	2	2	2	4	7	7	5	4	4	3	4	3	2	2	2	2	2	2	145	139	284	N	L	4	—	0/5	—	0/5	..	..
D2	–	6	6	4	4	3	2	3	3	..	3	2	2	4	4	4	4	4	3	2	3	3	3	3	3	3	3	3	48	234	282	N	L	2	—	0/5	—	0/5	..	..
D3	–	6	5	5	3	3	2	2	2	..	1	1	1	2	2	3	3	2	1	1	1	2	1	1	1	1	1	1	103	185	288	N	L	1	—	0/5	—	0/5	..	..
D4	–	8	6	6	5	4	3	3	2	..	3	2	3	4	4	2	3	3	3	3	4	2	3	3	3	3	3	2	153	134	287	N	L	3	—	0/5	—	0/5	..	..
D5	–	7	6	6	6	4	5	4	3	..	3	2	3	3	4	4	3	3	2	2	2	3	2	2	2	2	2	2	48	240	288	N	L	1	—	0/5	—	0/5	..	..
D6	–	8	7	4	6	5	4	4	3	..	3	3	3	7	7	8	9	9	9	9	9	8	7	7	6	6	6	6	124	87	211	PI	D	9	+	5/5	+	0/5	+	0/5
D7	–	5	5	4	4	4	5	4	2	..	2	1	1	3	3	3	2	2	1	–	–	–	–	–	–	–	–	–	122	157	279	N	L	–	—	0/5	—	0/5	..	..
D8	–	7	6	5	3	2	2	2	2	..	1	1	1	2	3	3	5	6	7	8	9	11	12	11	12	9	9	9	100	175	275	NI	L	10	+	5/5	+	4/4	..	..
D9	–	8	7	7	5	6	8	7	8	..	7	7	6	7	6	5	5	5	5	4	4	4	5	4	4	5	4	4	130	153	283	N	L	4	—	0/5	—	0/5	..	..
D10	–	7	7	6	4	5	4	3	3	..	2	3	3	7	4	4	3	2	3	3	4	3	2	2	2	2	3	3	131	110	241	PN-I	D	3	—	0/5	—	0/5	—	0/5

Agglutination tests, serum dilutions 1 = 1/10, 2 = 1/20, 3 = 1/40, 4 = 1/80, 5 = 1/160, 6 = 1/320, 7 = 1/640, 8 = 1/1,280, 9 = 1/2,560, 10 = 1/5,120, 11 = 1/10,240, 12 = 1/20,480. .. = not done. — = negative. + = positive. Cult. = Bacteriological examination. Biol. = Biological test, No. of guinea-pigs infected/No. used. P = premature. I = infected. N-I = Non-infected. N = Normal. L = Live. D = Dead.

agglutinins for *Br. abortus* were detectable in the blood and the cotyledons, and the foetal stomach and colostrum were proved to be free from infection by cultural and biological tests. It may, therefore, be concluded that the routine precautions taken to prevent the spread of infection between the units within the compound were adequate.

UNITS A AND E. *Effect, on non-vaccinated control and vaccinated animals, of exposure to the Small Infective Dose* (15 *million viable organisms.*)

The history of each animal is shown in detail in Tables I and II.

Animal A10 died of anthrax on 6.4.43 and E2 was barren ; thus, for the purpose of the experiment, the effective number of animals on the small test dose was reduced to nine controls and nine vaccinated.

AGGLUTINATION TITRES.—Table I shows that, apart from four occasions when titres of 1 in 10 were recorded, the control animals were negative to 13 agglutination tests during a period of 35 weeks prior to infection. A similar series of tests in the vaccinated animals showed that, following vaccination, maximum titres ranging from 1 in 320 to 1 in 1,280, with a median of 1 in 640 developed in about two to four weeks. Thereafter, the titres fell gradually until, at 35 weeks after vaccination, when the infective dose was applied, they ranged from 1 in 10 to 1 in 160 with a median of 1 in 40. Following exposure to infection the vaccinated animals developed a rise in titre and, excluding E5 which developed an active infection, maximum titres of 1 in 20 to 1 in 320, with a median of 1 in 120 developed in two to eight weeks. In the controls the rise in titres after infection was more gradual but reached a higher level than in the vaccinated animals and maximum titres of 1 in 640 to 1 in 20,480 with a median of 1 in 2,560 were reached in eight to 29 weeks.

INFECTION RATE.—Of the nine control animals all developed infection. *Br. abortus* was recovered from the cotyledons of nine out of nine cows ; from the colostrum of seven and from the contents of the foetal stomach in six out of six dead calves. Of the nine vaccinated animals, only one, E5, developed an active infection. The causal organism was not recovered by direct culture from this animal but the cotyledons and colostrum were found to be infected by biological test.

HISTORY OF PREGNANCY.—The duration of pregnancy in the controls ranged from 215 to 274 days with a mean of 246 days, while the range in the vaccinated group was 273 to 288 days with a mean of 280 days. Eight of the nine controls calved prematurely ; six of the calves were dead and three survived, whereas the nine vaccinated animals calved normally and produced nine living calves.

INFECTION RATE IN MILK.—*Br. abortus* was recovered from 70 out of 90 milk samples from control cows, but was not detected in any of the 90 samples from vaccinated cows (see Table II).

UNITS B AND D. *Effect, on non-vaccinated control and vaccinated animals, of exposure to the Large Infective Dose* (150 *million viable organisms*).

The history of each animal is shown in detail in Tables III and IV.

TABLE II
SMALL INFECTIVE DOSE—CONTROLS (UNVACCINATED)

Heifer No.	Post-partum milk samples examined at weekly intervals for *Br. abortus*																		Milk samples examined	
	1		2		3		4		5		6		7		8		9		10			
	Cult.	Biol.	Cult.	Biol.	Cult.	Biol.	Cult.	Biol.	Cult.	Biol.	Cult.	Biol.	Cult.	Biol.	Cult.	Biol.	Cult.	Biol.	Cult.	Biol.	Total	No. infected
A1	+	..	—	0/2	+	..	+	..	+	..	+	..	+	..	+	..	+	..	+	..	10	9
A2	—	0/2	+	..	—	2/2	+	..	—	0/2	+	..	—	0/2	+	..	—	0/2	—	0/2	10	5
A3	—	0/2	+	..	—	2/2	—	0/2	—	0/2	—	0/2	—	0/2	—	0/2	—	0/2	—	0/2	10	2
A4	+	..	+	..	+	..	—	2/2	+	..	+	..	—	2/2	—	0/2	+	..	+	..	10	9
A5	+	..	+	..	+	..	+	..	+	..	+	..	+	..	+	..	+	..	+	..	10	10
A6	+	..	+	..	+	..	—	2/2	—	1/2	+	..	—	1/2	+	..	+	..	—	1/2	10	10
A7	+	..	—	1/2	+	..	+	..	+	..	—	0/2	—	0/1	—	0/2	—	0/2	—	0/2	10	5
A8	+	..	+	..	+	..	+	..	+	..	+	..	+	..	—	2/2	+	..	+	..	10	10
A9	+	..	+	..	+	..	—	2/2	+	..	+	..	+	..	+	..	+	..	+	..	10	10
A10	Died of anthrax 6.4.43																					
																					90	70
SMALL INFECTIVE DOSE—VACCINATED GROUP																						
E1	—	0/2	—	0/2	—	0/2	—	0/2	—	0/1	—	0/2	—	0/2	—	0/2	—	0/2	—	0/2	10	0
E2	Not pregnant																					
E3	—	0/2	—	0/2	—	0/2	—	0/2	—	0/2	—	0/2	—	0/2	—	0/1	—	0/2	—	0/2	10	0
E4	—	0/2	—	0/1	—	0/2	—	0/2	—	0/2	—	0/2	—	0/2	—	0/2	—	0/1	—	0/2	10	0
E5	—	0/2	—	0/2	—	0/2	—	0/2	—	0/2	—	0/2	—	0/2	—	0/1	—	0/2	—	0/2	10	0
E6	—	0/2	—	0/2	—	0/2	—	0/2	—	0/2	—	0/1	—	0/2	—	0/1	—	0/2	—	0/2	10	0
E7	—	0/2	—	0/2	—	0/2	—	0/2	—	0/2	—	0/2	—	0/2	—	0/2	—	0/1	—	0/2	10	0
E8	—	0/2	—	0/2	—	0/2	—	0/2	—	0/2	—	0/2	—	0/2	—	0/2	—	0/2	—	0/2	10	0
E9	—	0/2	—	0/2	—	0/2	—	0/2	—	0/1	—	0/1	—	0/1	—	0/2	—	0/2	—	0/1	10	0
E10	—	0/2	—	0/2	—	0/2	—	0/2	—	0/2	—	0/2	—	0/2	—	0/2	—	0/2	—	0/1	10	0
																					90	0

TABLE IV
LARGE INFECTIVE DOSE—CONTROLS (UNVACCINATED)

Heifer No.	Post-partum milk samples examined at weekly intervals for *Br. abortus*																		Milk samples examined	
	1		2		3		4		5		6		7		8		9		10			
	Cult.	Biol.	Cult.	Biol.	Cult.	Biol.	Cult.	Biol.	Cult.	Biol.	Cult.	Biol.	Cult.	Biol.	Cult.	Biol.	Cult.	Biol.	Cult.	Biol.	Total	No. infected
B1	+	..	—	1/2	—	0/2	—	0/2	—	0/2	—	0/2	—	2/2	—	0/2	—	0/2	+	..	10	4
B2	—	1/2	—	0/2	+	..	+	..	—	0/2	—	0/2	—	0/2	—	0/2	—	0/2	—	0/2	10	3
B3	+	..	—	2/2	+	..	—	0/2	—	..	—	0/2	—	0/2	—	1/2	—	0/2	—	1/2	10	5
B4	+	..	—	0/2	—	2/2	—	1/1	+	..	—	0/2	—	0/2	—	0/2	—	..	—	0/2	10	4
B5	+	..	—	0/2	+	..	+	..	+	..	+	..	+	..	+	..	+	..	+	..	10	9
B6	+	..	—	1/2	—	0/2	+	..	—	0/2	—	1/2	—	0/1	—	0/2	—	2/2	—	0/2	10	5
B7	—	0/2	—	0/2	—	0/2	—	0/2	—	0/2	—	0/2	—	0/2	—	0/2	—	1/2	—	0/2	10	1
B8	+	..	+	..	+	..	+	..	+	..	+	..	+	..	+	..	+	..	—	0/2	10	9
B9	+	..	+	..	+	..	—	2/2	+	..	—	0/2	—	0/2	—	1/2	—	1/2	—	0/2	10	7
B10	+	..	+	..	+	..	+	..	+	..	+	..	+	..	+	..	—	0/2	—	0/2	10	8
																					100	55
LARGE INFECTIVE DOSE—VACCINATED GROUP																						
D1	—	0/2	—	0/2	—	0/2	—	0/2	—	0/2	—	0/2	—	0/2	—	0/2	—	0/2	—	0/2	10	0
D2	—	0/2	—	0/2	—	0/2	—	0/2	—	0/2	—	0/2	—	0/2	—	0/2	—	0/2	—	0/2	10	0
D3	—	0/2	—	0/2	—	0/2	—	0/2	—	0/2	—	0/2	—	0/2	—	0/2	—	0/2	—	0/2	10	0
D4	—	0/2	—	0/2	—	0/2	—	0/2	—	0/2	—	0/2	—	0/2	—	0/2	—	0/2	—	0/2	10	0
D5	—	0/2	—	0/2	—	0/2	—	0/2	—	0/2	—	0/2	—	0/2	—	0/2	—	0/2	—	0/2	10	0
D6	—	0/2	—	2/2	—	0/2	—	0/2	—	0/2	—	0/2	—	0/2	—	0/2	—	0/2	—	0/2	10	1
D7	—	0/2	—	0/2	—	0/2	—	0/1	—	0/1	—	0/2	—	0/2	—	0/1	—	0/2	—	0/2	10	0
D8	+	2/2	—	0/2	—	0/2	+	2/2	+	2/2	+	2/2	+	2/2	+	2/2	+	1/2	+	2/2	10	8
D9	—	0/2	—	0/2	—	0/2	—	0/2	—	0/2	—	0/2	—	0/1	—	0/2	—	0/2	—	0/2	10	0
D10	—	0/2	—	0/2	—	0/2	—	0/2	—	0/1	—	0/2	—	0/2	—	0/2	—	0/2	—	0/2	10	0
																					100	9

Agglutination Titres.—Table III shows that apart from five occasions when low titres were recorded, the control animals were negative to 13 agglutination tests during a period of 35 weeks prior to infection. A similar series of tests in the vaccinated animals showed that all were positive two weeks after vaccination and the maximum titres developed ranged from 1 in 160 to 1 in 1,280 with a median of 1 in 640. When the infective dose was applied the titres ranged from 1 in 10 to 1 in 320 with a median of 1 in 30. After applying the test dose the controls developed maximum titres in eight to 14 weeks ranging from 1 in 640 to 1 in 10,240 with a median of 1 in 2,560, while in the vaccinated group the maximum titres ranged from 1 in 40 to 1 in 20,480 with a median of 1 in 240.

Infection Rate.—All of the ten control animals developed infection. *Br. abortus* was recovered from the cotyledons of ten out of ten cows; from the colostrum of eight and from the contents of the foetal stomach in nine out of nine dead calves. Of the ten vaccinated animals, D10 aborted from a non-specific cause. Two animals, D6 and D8, developed an active infection and *Br. abortus* was isolated by direct culture from the cotyledons and colostrum of both. D6 had a premature dead calf and the contents of the foetal stomach were infected.

History of Pregnancy.—The duration of pregnancy in the controls ranged from 192 days to 263 days with a mean of 224 days, while in the vaccinated group (excluding D10) the range was 211 to 288 days with a mean of 275 days. All of the ten control animals calved prematurely, nine of the calves were dead and one survived, whereas in the vaccinated group (excluding D10) one animal only, D6, produced a premature dead calf, and the remaining eight had full-term living calves.

Infection Rate in Milk.—*Post-partum* milk samples examined weekly for ten weeks showed the presence of *Br. abortus* in 55 out of 100 samples from control animals and 9 out of 100 samples from vaccinated animals. Of the nine positive samples, eight were from the same animal, D8, and one from D6. Both of these animals failed to resist infection. (See Table IV.)

Character of Strains Recovered from Vaccinated Animals.—The strains isolated from vaccinated animals were all CO_2 sensitive and in that respect they resembled the infecting strain 544. Nine hundred and twenty-nine colonies of *Br. abortus* obtained by direct culture, from the two vaccinated animals, D6 and D8, that failed to resist infection, were examined for their sensitivity to the bacteriostatic action of thionine blue by the method described by McLeod (1944) and all were capable of growth on a medium containing a concentration of thionine blue which inhibited the growth of strain 19. Further, of 558 guinea-pigs used for the biological tests of material from all the vaccinated animals, 39 inoculated with material from the three vaccinated animals, D6, D8 and E5, developed infection. The disease in these guinea-pigs was typical of that produced by a fully virulent strain, with enlargement and heavy infection of the spleen six weeks after injection.

For convenience of interpretation the experiment is summarized in Table V.

Table V

Strain 19 Vaccine

Summary of Important Points of Difference between Vaccinated and Control Cattle exposed during Pregnancy to Infection with *Brucella abortus*, Strain 544

Infective dose applied 35 weeks after vaccination

		Range and median of maximum agglutination titres for 35 weeks after vaccination and prior to infection	Range and median of agglutination titres at the time of applying infection	Range and median of maximum agglutination titres after applying infection	Range and mean duration of pregnancy in days	Number of animals which developed infection	Number of living calves per pregnant females	Number of milk samples with *Br. abortus* during 10 weeks after parturition
Small infective dose ..	Vaccinated	1/320 to 1/1,280 Median 1/640	1/10 to 1/160 Median 1/40	1/20 to 1/320 Median 1/120	273 to 288 Mean 280	1 out of 9	9 out of 9	0 out of 90
15 million viable organisms	Controls	0 to 1/10	0 to 1/10	1/640 to 1/20,480 Median 1/2,560	215 to 274 Mean 246	9 out of 9	3 out of 9	70 out of 90
Large infective dose ..	Vaccinated	1/160 to 1/1,280 Median 1/640	1/10 to 1/320 Median 1/30	1/40 to 1/20,480 Median 1/240	211 to 288* Mean 275	2 out of 10	8 out of 9	9 out of 100
150 million viable organisms	Controls	0 to 1/20	0 to 1/10	1/640 to 1/10,240 Median 1/2,560	192 to 263 Mean 224	10 out of 10	1 out of 10	55 out of 100

* Excludes D10 which aborted from a cause other than infection with *Br. abortus*.

Comparison of virulence and immunological properties of Strain 19 and Strain 45/20

It is generally accepted that the incidence of contagious abortion in cattle can be reduced by the use of vaccines containing living *Br. abortus*. When such vaccines contain fully virulent organisms, however, there is always the risk of establishing a persistent infection in the animal body and especially in the udder. There has been objection, therefore, to the use of virulent living vaccines in non-infected herds because of the danger of spreading the infective agent and the risk of infecting the consumer of milk from vaccinated animals. Attempts have been made to eliminate this danger by the use of vaccines prepared from strains of reduced virulence, which are less liable to cause persistent infection in the animal body. Prominent amongst these is strain 45/20 (McEwen, 1937 to 1946) which has been extensively used in this country and issued until recently by the Ministry of Agriculture as abortion vaccine No. 2, and strain 19 (Buck, 1930) used extensively in the United States (Huddleson, 1942), (Crawford, 1944), and issued in this country by the Ministry of Agriculture as abortion vaccine No. 1.

In selecting one of a number of avirulent strains for immunization of cattle on a nation-wide scale it is, in the first place, desirable to choose the strain that produces the best immunity and which, at the same time, is least likely to cause a persistent infection. In the second place, in the interests of safeguarding public health, preference would be given to the strain in which the character of reduced virulence is most stable. It is appropriate, therefore, to compare the evidence so far obtained regarding the virulence, stability and immunizing properties of strain 19 and strain 45/20.

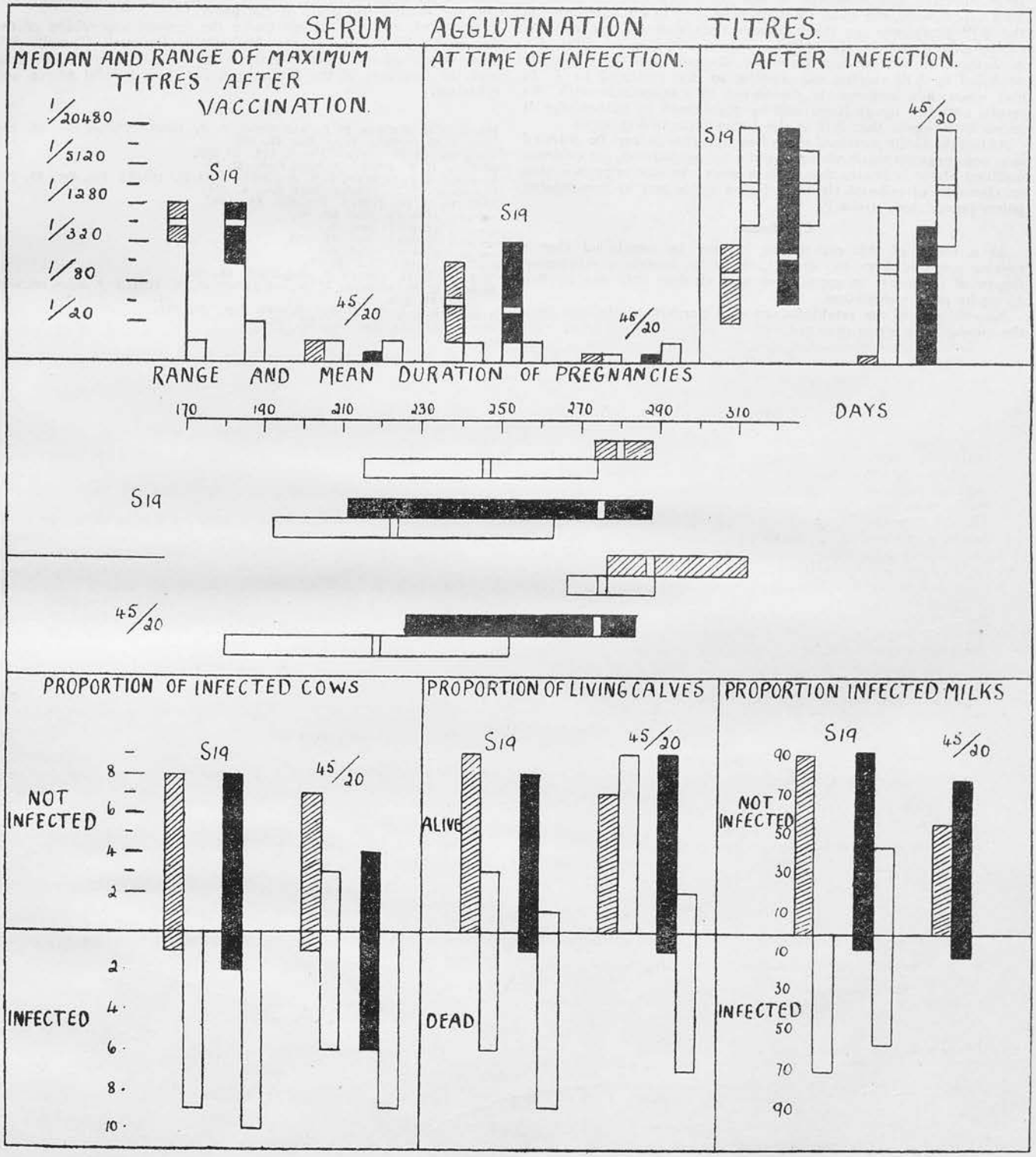
In the guinea-pig, de Ropp (1945) showed that strain 45/20 is more virulent since it persists in the spleen for a period up to 12 weeks, whereas strain 19 is eliminated in less than six weeks. He also found that vaccines prepared from strain 19 produced a better immunity in guinea-pigs than vaccines prepared from 45/20. Stableforth (1945) has also reported that in guinea-pigs strain 19 gives a significantly higher protection than strain 45/20.

In the bovine, McEwen (1940) found that strain 45/20, which is a serologically rough type, caused abortion and reverted to a smooth virulent form when injected into animals well advanced in pregnancy. A susceptible pregnant animal which was in contact aborted and an aerobic strain of *Br. abortus*, believed to be derived from the vaccine strain, was isolated from the foetus and the milk. This enhancement of virulence was also observed by passage of the strain through guinea-pigs. When non-pregnant cattle were inoculated with strain 45/20, however, McEwen (1940a, 1940b and 1946) found no evidence of the persistence of the organism, whereas Edwards, de Ropp and McLeod (1945) could not exclude the possibility that the vaccine strain might become enhanced in virulence and persist for long periods in the animal body.

Mingle, Manthei and Jasmin (1941) passaged strain 19 through a series of guinea-pigs without causing any change in its original character. Extending these investigations still further, they found it possible by injection of large doses intravenously, to induce abortion in pregnant cattle with strain 19, but no evidence of spread of infection to susceptible cattle kept in contact was obtained. Brucella cultures recovered from aborted foetuses were injected in series into pregnant cattle for seven generations. Critical comparison of the cultures recovered at successive generations revealed no detectable changes either in virulence or other characters. Further evidence of the stability of the character of reduced virulence after passage through the pregnant cow has been presented by Birch, Gilman and Stone (1943).

A comparison of the agglutination response and immunity produced in cattle by means of the two vaccines is set out in the accompanying diagram. This has been prepared from the data in the present paper relating to S 19 and from the paper by Edwards *et al.*, relating to 45/20. In making this comparison, attention is directed to the following differences in the two experiments. Firstly, the cattle vaccinated with 45/20 received two doses, each of 8·0 ml. at a month's interval, representing 240,000 million organisms, whereas those vaccinated with S 19 received a single dose of 60,000 million organisms. Thus the 45/20 group had the

COMPARISON OF REACTION IN CATTLE VACCINATED WITH S19 AND 45/20 WHEN EXPOSED TO INFECTION ALONG WITH CONTROLS.



benefit of a double stimulus with four times the number of organisms given to the S 19 groups as a single stimulus. Secondly, while the large infective dose employed to test immunity was the same in both experiments, the small infective dose used to test immunity in the S 19 group was ten times greater than that used to test the comparable group in the 45/20 experiment. Despite these disadvantages, it will be seen from the diagram that the immunity produced by S 19 vaccine was superior to that produced by 45/20 and when this evidence is considered in conjunction with the results obtained by de Ropp and by Stableforth in guinea-pigs, it leaves little doubt that S 19 is the better immunizing agent.

Although 45/20 possesses the advantage that it can be injected into non-pregnant cattle without producing agglutinins, the evidence outlined above indicates that it is a more virulent organism, that its character of reduced virulence is less stable and its immunizing power poorer than strain 19.

Conclusion

As a result of this experiment it may be concluded that a vaccine prepared from *Br. abortus*, strain 19, confers a substantial degree of immunity in cattle against a virulent infection applied 35 weeks after vaccination.

No evidence of the establishment of a persistent infection from the vaccine strain was obtained.

Acknowledgments.—We would like to take this opportunity of expressing our gratitude to Dr. W. S. Gordon, Director of this Station, for his constant advice in the direction of this experiment and for writing the draft of the paper.

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The Transference of Agglutinins for Brucella Abortus from Cow to Calf and their Persistence in the Calf's Blood

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It has been shown that all calves are born without agglutinins for *Br. abortus*, regardless of the reaction of their dams (McAlpine and Rettgar, 1925) but that such agglutinins are almost invariably demonstrable after calves have fed on the colostrum of infected cows (Little and Orcutt, 1922) and disappear within a period of six months (Thorp and Graham, 1933).

In this paper further evidence is presented regarding the interval between the ingestion of colostrum and the detection of agglutinins in the blood of the calf, and the curve determining the disappearance of these agglutinins has been established.

Technique

ANIMALS EMPLOYED.—Sixteen Ayrshire and Friesian heifers, which were being used to test the immunizing value of S.19 vaccine (Edwards, McDiarmid, de Ropp and McLeod, 1946) were employed, 12 of the heifers had been vaccinated and all had been exposed to a virulent infection during pregnancy. The individual histories are shown in Table I.

METHODS OF OBTAINING SAMPLES

(*a*) *Colostrum.*—The udder was washed, dried and the teats swabbed with 50 per cent. alcohol. The first three jets of milk from each quarter were discarded. Thereafter a sterile McCartney bottle was filled with approximately 5 ml. of milk from each quarter. The samples were retained in the cold store until examined.

(*b*) *Milk.*—The samples were obtained in the same manner as above except that the udder was practically milked out prior to withdrawing the samples.

(*c*) *Blood.*—Samples were taken from the jugular vein.

CULTURAL EXAMINATION OF THE MILK AND COLOSTRUM.—0·5 ml. of milk or colostrum was spread on the surface of a freshly prepared 2½ per cent. ox blood bacto-tryptose agar plate. The plate was incubated in 10 per cent. CO_2 for five days at 37° C.

IDENTIFICATION OF *Br. abortus*.—A film was made from the suspected colony and stained for a few seconds with undiluted carbol fuchsin. A slide agglutination test was also prepared. If the organisms resembled *Br. abortus* culturally, morphologically and the agglutination test proved positive, the colony was accepted as *Br. abortus*.

AGGLUTINATION TEST.—Colostrum and milk samples were treated with rennet and the resultant whey was centrifuged to obtain a clear fluid for the agglutination test. The blood samples were kept at room temperature overnight, then centrifuged and the sera and whey tested by the technique described by Stableforth (1936) using normal saline for preparing the dilutions and the standard antigen kindly supplied by the Ministry of Agriculture Laboratory, Weybridge. Readings were taken after overnight incubation at 37° C., the titre recorded being the highest dilution of serum or whey which produced visible agglutination.

Experimental

At birth, the calf was prevented from sucking until the following samples had been obtained:—

1. Blood from dam for agglutination test.
2. Blood from calf for agglutination test.
3. Colostrum from dam for cultural examination and whey agglutination test.

TABLE I

INDIVIDUAL HISTORIES

No. of Heifer	Breed	Service date	Infected with *Br. abortus*, 13.4.43	Date of parturition	Gestation period	Parturition	Description of calf
INFECTED CONTROL GROUP							
A1	B.F.	30.10.42	15 million virulent organisms (Strain 544)	22.7.43	265	Premature +	Apparently normal (M.)
A2	A.	8.11.42	" " (")	28.7.43	262	" +	" " (M.)
A5	A.	23.11.42	" " (")	24.8.43	274	Normal +	" " (F.)
B7	A.	19.10.42	150 million virulent organisms (Strain 544)	9.7.43	263	Premature +	Very weak, unable to suck unaided (F.)
VACCINATED GROUP*							
D3	A.	31.12.42	150 million virulent organisms (Strain 544)	15.10.43	288	Normal	Normal (M.)
D4	A.	11.11.42	" " (")	25.8.43	287	"	" (M.)
D5	A.	24.2.43	" " (")	9.12.43	288	"	" (M.)
D7	A.	12.12.42	" " (")	17.9.43	279	"	" (F.)
D8	A.	3.1.43	" " (")	5.10.43	275	" +	Apparently normal (F.)
D9	B.F.	4.12.42	" " (")	13.9.43	283	"	Normal (M.)
E1	A.	21.11.42	15 million virulent organisms (Strain 544)	26.8.43	278	"	" (M.)
E3	A.	15.11.42	" " (")	18.8.43	276	"	" (F.)
E4	A.	31.10.42	" " (")	15.8.43	288	"	" (M.)
E7	B.F.	18.11.42	" " (")	26.8.43	281	"	" (M.)
E9	A.	18.12.42	" " (")	22.9.43	278	"	" (F.)
E10	A.	2.12.42	" " (")	10.9.43	282	"	" (F.)

B.F. signifies British Friesian.
A. " Ayrshire.
\+ " *Br. abortus* isolated from foetal membranes

* signifies vaccinated subcutaneously with 5 ml. S.19 vaccine 13.8.42.
(M.) " Male.
(F.) " Female.

Table 11

The Mode of Transference of Agglutinins for *Brucella abortus* from Cow to Calf

Agglutination titre of cow at parturition, culture of colostrum, and agglutination titre of whey and cultural examination of milk after parturition (weeks)

No. of cow	Blood	Whey	Culture of colostrum	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Infected Control Group																							
A1	7	10	N	+ 4	N 4	+ 4 (8)	+ 4	+ 4	+ 4 (8)	+ 5	+ 5	+ 5 (10)	+ 4	5	5 (8)	5	3	5 (10)	4	5	5	4	
A2	6	11	N	N 6	+ 6 (8)	N 5	+ 5	N 5 (8)	+ 5	N 5	+ 6 (8)	N 6	N 6	6 (6)	4	3	5 (6)	4	4	3	3		
A5	7	10	+	+ 5 (6)	+ 5	+ 4	+ 5 (9)	+ 4	+ 5	+ 6 (8)	+ 5	+ 5	+ 5 (9)	5	5	4	4	3					
B7	7	9	N	N 3	N 2 (7)	N –	N	N (6)	N	N	N (5)	N	N	(3)									
Vaccinated Group																							
D3	1	3	N	N –	N	N (1)	N	N	N (1)	N	N	N (1)	N		(1)			(1)			(1)		
D4	3	5	N	N – (3)	N	N	N (3)	N	N	N (4)	N	N	N (2)			(3)			(3)			(3)	
D5	2	4	N	N – (2)	N	N	N (2)	N	N	N (2)	N	N	N (2)										
D7	–	3	N	N –	N	N (–)	N	N	N (–)	N	N	N (–)	N		(–)			(–)			(–)		
D8	10	11	+	+ 5 (11)	N 5	N 5	+ 4 (12)	+ 6	+ 4	+ 4 (11)	+ 3	+ 4	+ 4 (12)	3	–	(9)			(9)			(9)	
D9	4	9	N	N 1 (4)	N	N	N (4)	N	N	N (4)	N	N	N (5)			(4)			(4)			(5)	
E1	4	5	N	N – (4)	N	N	N (4)	N	N	N (3)	N	N	N (2)			(2)							
E3	4	7	N	N –	N (3)	N	N	N (3)	N	N	N (3)	N	N	(2)			(3)						
E4	6	10	N	N –	N (5)	N	N	N (5)	N	N	N (5)	N	N	(4)			(4)						
E7	4	8	N	N – (4)	N	N	N (4)	N	N	N (5)	N	N	N (3)			(4)							
E9	3	5	N	N –	N	N (2)	N	N	N (2)	N	N	N (2)	N										
E10	5	8	N	N 4	N 2 (5)	N	N	N (5)	N	N	N (4)	N	N	(4)									

Interval between birth and sucking in hours, and agglutination titre of the calves' blood (before sucking; after sucking — mins., hours, weeks)

No. of cow	Interval (hours)	Before sucking	5 min	10 min	15 min	1 h	2 h	3 h	24 h	48 h	72 h	Wk 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Infected Control Group																															
A1	1	–	–	–	–	–	3	3	9	8	8	8	7	6	6	3	4	4	4	4	3	3	1	2	1	1	1	1	1	–	
A2	3·5	–	–	–	–	–	3	3	7	7	7	6	6	6	5	4	3	4	3	3	3	2	2	2	2	2	1	1	–		
A5	1	–	–	–	–	–	–	4	8	8	7	6	5	5	6	6	6	4	4	4	3	3	3	2	1	–					
B7	2·5	–	–	–	–	–	S	2	5	5	4	4	3	3	3	3	2	–													
Vaccinated Group																															
D3	6	–	–	–	–	–	–	–	–	–	–	–																			
D4	7	–	–	–	–	–	–	–	1	2	2	–																			
D5	9·5	–	–	–	–	–	–	1	2	2	1	–																			
D7	6	–	–	–	–	–	–	1	2	2	1	–																			
D8	7	–	–	–	–	3	6	8	10	9	9	9	12	10	7	9	8	7	6	5	5	3	1	–							
D9	1	–	–	–	–	–	–	1	2	3	3	2	–																		
E1	11	–	–	–	–	–	–	–	1	–	–	–																			
E3	15·5	–	–	–	–	–	–	1	3	2	2	1	–																		
E4	12	–	–	–	–	–	2	3	4	4	5	3	–																		
E7	6	–	–	–	–	–	–	5	7	6	6	4	3	3	3	3	3	1	–												
E9	10·5	–	–	–	–	–	–	1	3	3	3	1	–																		
E10	6·5	–	–	–	–	–	3	4	6	6	5	4	3	3	2	2	2	2	1	1	–										

N Signifies no *Br. abortus* isolated.
\+ ,, *Br. abortus* isolated.
– Negative agglutination titre.
S ,, Sample not obtained.

( ) Signifies Blood agglutination titre of dam.
The agglutination titre is indicated by a number which refers to the last tube in which visible agglutination occurred.
The dilutions of serum were as follows : 1 : 10, 1 : 20, 1 : 40, etc.

FIG. I.

A 2.

THE TITRE OF THE CALF'S BLOOD AND THAT OF THE WHEY AND BLOOD OF THE DAM.

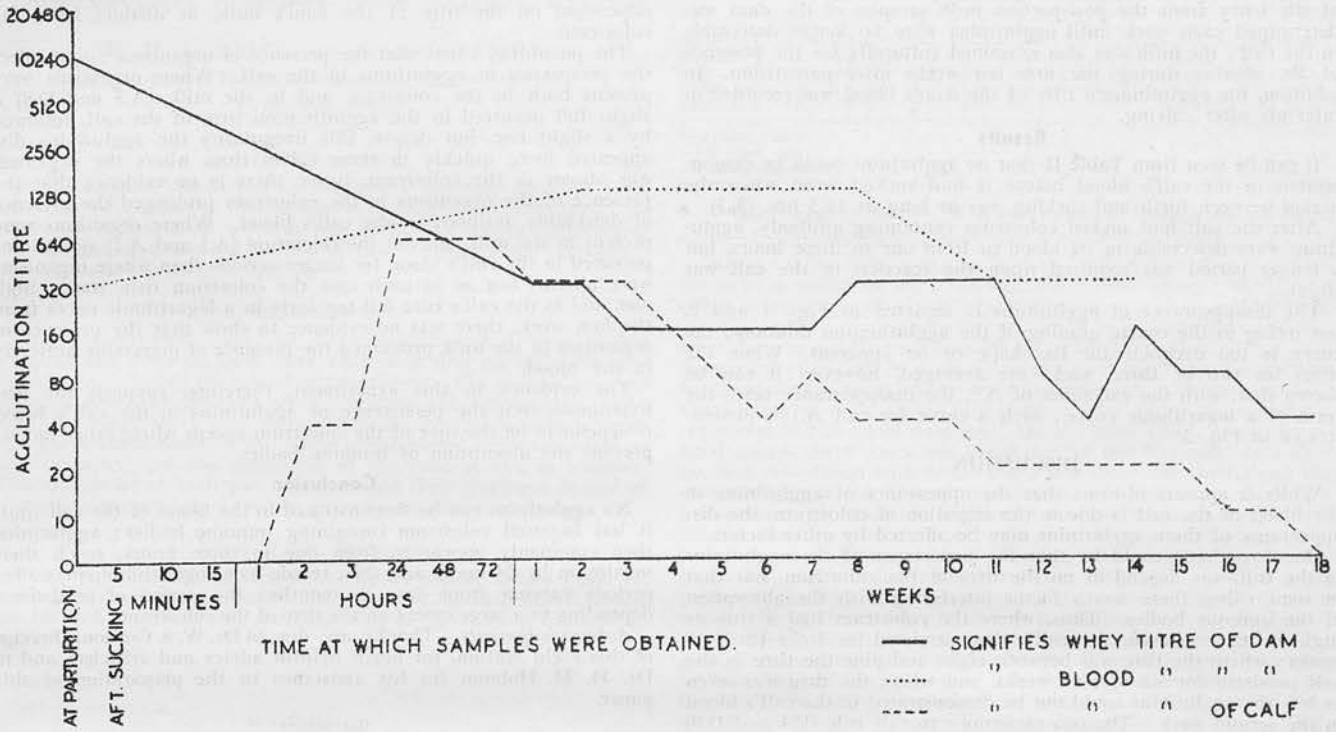


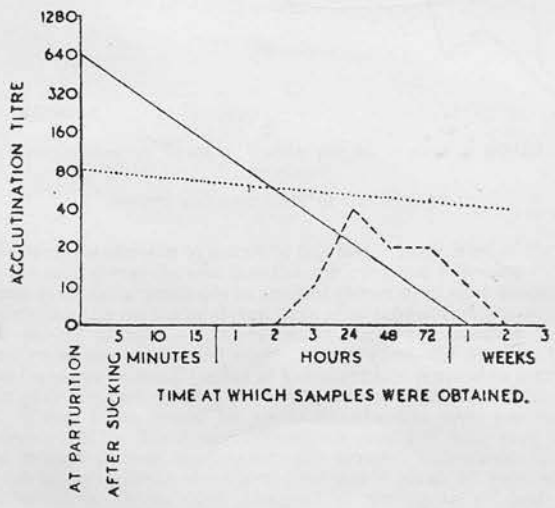
FIG. 2.

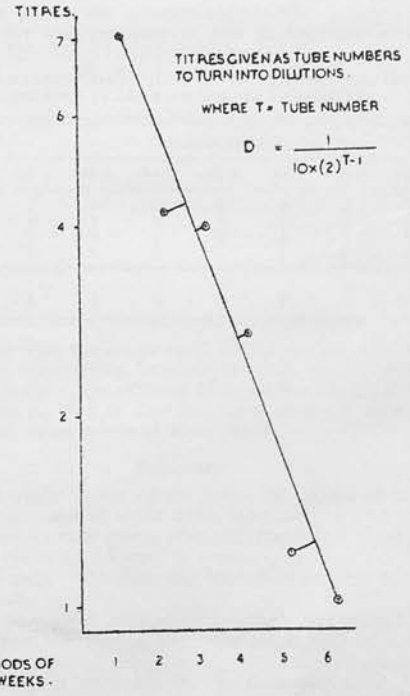
E 3.

FIG. 3.

CURVE FOR DISAPPEARANCE OF AGGLUTININS IN CALF'S BLOOD.

THE TITRE OF THE CALF'S BLOOD AND THAT OF THE WHEY AND BLOOD OF THE DAM.





AVERAGE TITRES FOR 3 WEEK PERIODS ON LOGARITHMIC SCALE CALF A.I.
(LINE DRAWN FREEHAND.)

Thereafter the calf was allowed to take a substantial feed of its own mother's colostrum and, in most cases, blood samples were obtained from the calf at intervals of five, ten and 15 minutes, one, two, three, 24, 48 and 72 hours and then weekly until agglutinins were no longer detectable. After the first blood sample had been obtained, the calf was allowed to suck its own mother at will throughout the course of the experiment. The agglutination titre of the whey from the *post-partum* milk samples of the dam was determined each week until agglutinins were no longer detectable in the calf; the milk was also examined culturally for the presence of *Br. abortus* during the first ten weeks after parturition. In addition, the agglutination titre of the dam's blood was recorded at intervals after calving.

Results

It can be seen from Table II that no agglutinins could be demonstrated in the calf's blood before it had sucked, even when the period between birth and sucking was as long as 15·5 hrs. (E.3).

After the calf had sucked colostrum containing antibody, agglutinins were detectable in its blood in from one to three hours, but a longer period was required when the reaction in the calf was slight.

The disappearance of agglutinins is depicted in Figs. 1 and 2, but owing to the coarse grading of the agglutination dilutions, the curve is too irregular for its shape to be apparent. When the titres for two or three weeks are averaged, however, it can be shown that, with the exception of A.5, the disappearance takes the form of a logarithmic curve; such a curve for calf A.1 is demonstrated in Fig. 3.

DISCUSSION

While it appears obvious that the appearance of agglutinins in the blood of the calf is due to the ingestion of colostrum, the disappearance of these agglutinins may be affected by other factors.

One hypothesis could be that the persistence of the agglutinins in the calf was dependent on the titre of the colostrum, but that in some calves there was a factor interfering with the absorption of the immune bodies. Thus, where the colostrum had a titre as high as ten or eleven, the calf's titre persisted for from 12 to 18 weeks; where the titre was between eight and nine the titre in the calf persisted for six to nine weeks, and where the titre was seven or less the agglutinins could not be demonstrated in the calf's blood in the second week. The two exceptions to this rule (E.4 and D.9) would, with this hypothesis, be accepted as cases in which there was some failure in the absorption of immune bodies.

The persistence of agglutinins in the blood of the calf as compared to the whey of the dam is shown in Figs. 1 and 2. The investigation of a possible correlation between these two titres was weakened by the fact that the estimation of the whey agglutinins was discontinued as soon as the calf's serum became negative. It could be said, however, that where the whey agglutinins were persistent, the sera agglutinins also persisted, but the converse does not hold good, for in Heifer E.7 the whey was negative and yet agglutinins peristed in the calf for seven weeks. There is, therefore, no evidence that the agglutination titre of the calf is dependent on the titre of the dam's milk, as distinct from the colostrum.

The possibility exists that the presence of organisms might affect the persistence of agglutinins in the calf. Where organisms were present both in the colostrum and in the milk (A.5 and D.8) a slight fall occurred in the agglutination titre of the calf, followed by a slight rise, but despite this irregularity the agglutinins disappeared more quickly in these calves than where the organism was absent in the colostrum, hence there is no evidence that the presence of the organisms in the colostrum prolonged the presence of detectable antibody in the calf's blood. Where organisms were present in the milk but not the colostrum (A.1 and A.2) agglutinins persisted in the calf's blood for longer periods than where organisms were absent, but as in each case the colostrum titre was a high one, and as the calf's titre fell regularly in a logarithmic curve from the first week, there was no evidence to show that the presence of organisms in the milk prolonged the presence of detectable antibody in the blood.

The evidence in this experiment, therefore, supports the first hypothesis, that the persistence of agglutinins in the calf's blood is dependent on the titre of the colostrum except where other factors prevent the absorption of immune bodies.

Conclusion

No agglutinins can be demonstrated in the blood of the calf until it has ingested colostrum containing immune bodies; agglutinins then commonly appear in from one to three hours, reach their maximum by 24 hours and then recede as a logarithmic curve over periods varying from days to months; the period of persistence depending to a large extent on the titre of the colostrum.

Acknowledgments.—Thanks are due to Dr. W. S. Gordon, Director of this Field Station, for much helpful advice and criticism and to Dr. H. H. Holman for his assistance in the preparation of this paper.

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An Observation on Reduction in Milk Yield following Vaccination of Lactating Cows with Living Vaccines Prepared from Brucella Abortus

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INTRODUCTION

It is common experience to observe a decrease in milk yield following prophylactic vaccination in a dairy herd. During the course of experimental work at this Field Station, an opportunity was afforded of measuring the loss of milk resulting from vaccination with *Brucella abortus* live vaccine.

EXPERIMENTAL

Observations were made on 29 tuberculin-tested, mastitis-free Ayrshire and Friesian dairy cows after their first calving. No cow was pregnant during the period of experiment. Six animals received 5 ml. of strain 19 *Brucella abortus* vaccine subcutaneously; eight were inoculated twice with strain 45/20 *Br. abortus* vaccine with an interval of three weeks, 4 ml. being administered subcutaneously on each occasion, and the remainder of the herd served as controls. The milk yield of each cow was recorded daily during a period of 14 weeks commencing six weeks prior to the first inoculation.

RESULTS AND DISCUSSION

The results are summarised in the figure and in Tables I and II. The figure shows the weekly yield per cow for each group, and it can be seen that following each inoculation there was a decrease in yield.

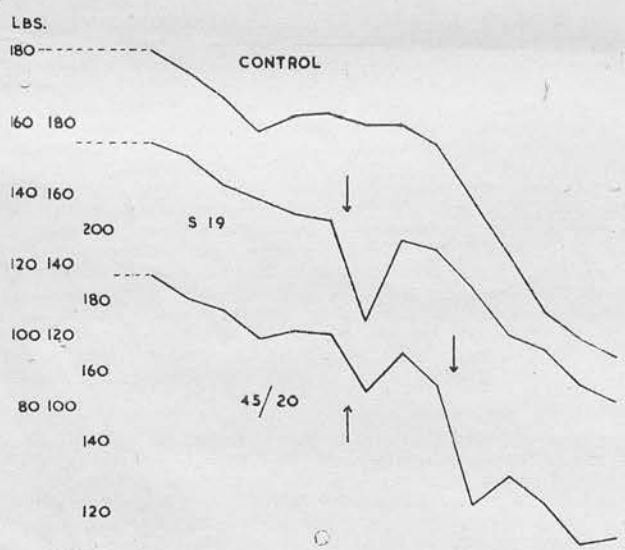

FIG.—Comparison of Weekly Yields per Cow over a period of 14 weeks.

Arrows indicate time of inoculation.

An attempt was made to calculate this loss. Inspection of the daily totals for each group showed that the apparent loss following inoculation was in all cases negligible in amount eleven days after inoculation, and therefore the period of eleven days after inoculation was accepted as the period of loss. The regression curve for lactation is considered to be an exponential curve, nevertheless, the error in taking the decrease over a small period as a straight line regression is trifling. To simplify the calculations, therefore, straight line regressions were used. These lines, based on yields for the five days previous to inoculation and for five days following the period of loss, were drawn by the method of least squares for each group. To estimate the loss incurred by inoculation the theoretical yields given by this line for the intervening eleven days, accepted as the period of loss, were subtracted from the actual yields for the same period, and the results are recorded in Table I.

TABLE I.—DAILY LOSSES PER COW IN LB.

	Cows in Group	Days after Inoculation 1	2	3	4	5	6	7	8	9	10	11	Total
Control ..	15	0·6	0·2	+0·5	+0·1	+0·1	0·6	1·2	1·5	0·3	0·5	0·5	4·7
S.19 ..	6	0·7	7·0	6·7	4·8	3·2	3·2	2·7	2·2	0·8	1·7	0·5	33·5
45/20. 1st	8	1·5	4·9	2·6	1·6	1·3	1·6	1·6	1·1	+0·1	0·1	+0·4	15·8
45/20. 2nd	8	0·2	4·5	3·7	4·0	4·6	3·7	0·5	1·0	0·6	0·4	1·4	24·6
Weighted means for loss per inoculated cow ..		0·8	5·3	4·1	3·4	3·0	2·8	1·5	1·4	0·4	0·6	0·5	24·0*

\+ = Gain instead of loss. * = total dependent on three decimal places.

Owing to the large variation in daily milk yields that occurs in the cow, it would be expected that the control group would show either a small loss or a small gain when compared to a theoretical standard, and in this case there was a small loss amounting to 4·7 lb., equivalent to a difference of 1·86 per cent. between the actual and theoretical yields, while a chi squared test showed that the relative frequencies of gains and losses were homogeneous with a hypothetical distribution of daily yields falling above and below the calculated regression line in equal numbers. On the other hand, in the inoculated groups there was a loss every day for the first eight days after the first inoculation with 45/20 and a loss every day throughout the eleven days for the second inoculation, and following the single inoculation with strain 19. Following the first inoculation with 45/20 the loss was less than with strain 19, but the sum of the losses due to the two inoculations with 45/20 was greater than the loss due to strain 19. Little reliance, however, can be placed on these comparative figures owing to the great variation among individual cows, which will be shown later, and it is preferable to group the results of all three inoculations together and examine the weighted means giving the daily loss per inoculated cow. The cows were inoculated on Monday morning, and it can be seen that there was a small loss for the 24 hours that included the afternoon milking on Monday and the morning milking on Tuesday; during the next three periods of 24 hours the loss was at its maximum with a mean of 4·2 lb., it then receded to become negligible nine days after inoculation. The average total loss per cow amounted to 24 lb.

To assess the total loss for each individual cow advantage was taken of the fact that a straight line regression is a form of arithmetical progression, so that the sum of the first and last terms equals the mean. Hence the loss can be calculated on the basis that the mean from the five daily yields previous to inoculation plus the five daily yields following the period of loss should equal the mean obtained from the yields during the intervening period.

The results for the variation in loss in the individual cow are given in Table II.

TABLE II.—FREQUENCY DISTRIBUTION OF TOTAL LOSSES FOR INDIVIDUAL COWS OVER 11 DAYS FOLLOWING INOCULATION

	Losses in Lb. −78·5	−66·5	−54·5	−42·5	−30·5	−18·5	−6·5	Gains +6·5
Control ..	0	0	0	0	0	2	9	4
S.19	0	1	0	3	0	1	1	0
45/20. 1st ..	0	0	0	0	1	5	2	0
45/20. 2nd ..	1	0	0	0	2	2	3	0
Totals of inoculated cows ..	1	1	0	3	3	8	6	0

It can be seen that the losses vary widely among cows and do not permit a reliable comparison between the different injections. Eight out of 22 cows made a loss of from 13 to 24 lb. and fell into the class with a mid-point of 18·5 lb. but the curve shows a skew to the left with two cows showing a loss of 60 or 80 lb.

Summary

1. The daily milk yields of six cows inoculated with strain 19 *Br. abortus* vaccine and of eight cows inoculated with two doses of 45/20 *Br. abortus* vaccine given with an interval of three weeks are compared with those of 15 control cows.

2. A loss in milk yield followed inoculation; this loss averaged at 24 lb. per cow.

3. The loss was most pronounced during the period from two to four days after inoculation and was negligible nine days after inoculation.

4. There was great variation in the estimated total loss among individual cows, and estimates varied from a loss of 0·3 lb. to a loss of 80 lb. with a modal loss of 18·5 lb.

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A Comparison of the Immunity Produced in Guinea-pigs by the Inoculation of S.19 Br. abortus Vaccine Intradermally and Subcutaneously

By

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Introduction

Recent work by Rabstein and Cotton (1943) and Campbell and Rodwell (1945) has shown that a small dose, such as 0·2 ml., of the standard S.19 *Br. abortus* vaccine administered intradermally in cattle, produces an agglutination response similar to that obtained by the inoculation of 5 ml. subcutaneously, whereas 0·2 ml. inoculated subcutaneously does not produce such a high titre as the 5 ml. dose. Such a method of intradermal vaccination would have advantages over the more commonly employed subcutaneous method, because of the economy in the quantity of living antigen required to produce immunity, and an experiment is now in progress at this Field Station to compare the resistance of heifers vaccinated by both methods to a test dose of virulent *Br. abortus* during pregnancy.

Methods and Materials

Experimental Animals.—Guinea-pigs of either sex and of about 250 grammes in weight, were used.

Dosage and Route of inoculation

1. Intradermal.

The skin was depilated with barium sulphide and 0·2 ml. of a 1 in 5 dilution of S.19 vaccine was injected in the flank.

2. Subcutaneous.

The inoculum consisted of 1 ml. of undiluted vaccine and the point of inoculation was the same as that described above.

The S.19 vaccine was supplied by the Ministry of Agriculture Veterinary Laboratory at Weybridge and consisted of a sample of the routine issue.

Agglutination Tests.—Blood was obtained by cardiac puncture and tests were prepared according to the technique of Stableforth (1936). The end point was taken as the last tube to show visible signs of agglutination.

Infective Dose.—A dried culture of virulent *Br. abortus* (Strain 544, McEwen, 1940) was reconstituted with Hartley's broth, grown on bactotryptose agar slopes for 72 hours at 37° C. and then subcultivated on the same type of medium for a further 48 hours. This culture was washed off with Ringer solution and standardised to an opacity matching Brown's tube 10; dilutions were prepared from this suspension so that 1 ml. of the diluted material contained approximately 3,000,000 viable *Br. abortus* organisms (de Ropp, 1945). This number was checked by plate counts. 1 ml. of this diluted material was administered intramuscularly.

Post-mortem examination of guinea-pigs for *Br. abortus* infection

The criteria of infection were similar to those employed by de Ropp (1945) and consisted of the agglutination titre at the time of killing, the spleen weight, expressed as a percentage of the body weight, and the presence or absence of *Br. abortus* in the spleen. The spleen cultures were prepared by emulsifying the whole spleen in twice its own volume of Ringer solution and spreading 0·1 ml. of the suspension on two plates of bactotryptose blood agar.

Experimental Procedure

Forty-five guinea-pigs were divided into three equal groups.

Group 1. Vaccinated subcutaneously.
Group 2. Vaccinated intradermally.
Group 3. Non-vaccinated controls.

All the guinea-pigs were bled at weekly intervals. The infective dose was applied to all 45 guinea-pigs six weeks after the vaccination of groups one and two. Thereafter five guinea-pigs from each group were killed at three, seven and 15 weeks respectively and the degree of infection in each animal assessed.

Results and Discussion

Fig. 1 shows the mean agglutination titres produced in each group. The titres shown after the infective dose was applied are limited to the controls and to those guinea-pigs which resisted infection in the other two groups. Fig. 2 illustrates the degree of infection, the mean agglutination titres and the mean spleen weight/body weight ratios in each group at the time of killing. Fig. 1 shows that a close similarity existed between the agglutination titres produced by subcutaneous and intradermal inoculation and that the end point reached, 15 weeks after infection was applied, was identical following both methods of vaccination.

MEAN AGGLUTINATION TITRES
OF IMMUNE VACCINATED GUINEA PIGS AND NON VACCINATED CONTROLS

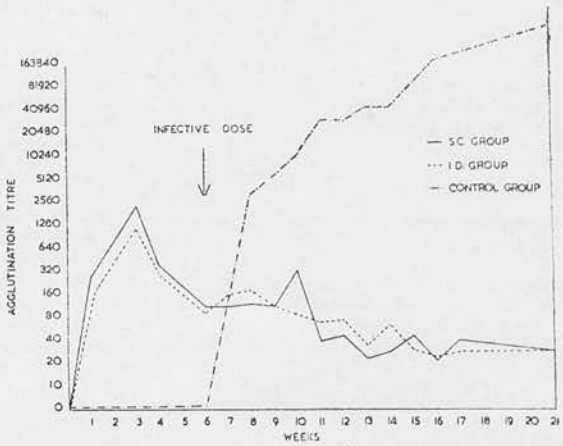

Fig. 1.

THE DEGREE OF INFECTION TITRE & SPLEEN WEIGHT BODY WEIGHT RATIO
IN VACCINATED AND NON VACCINATED GUINEA PIGS

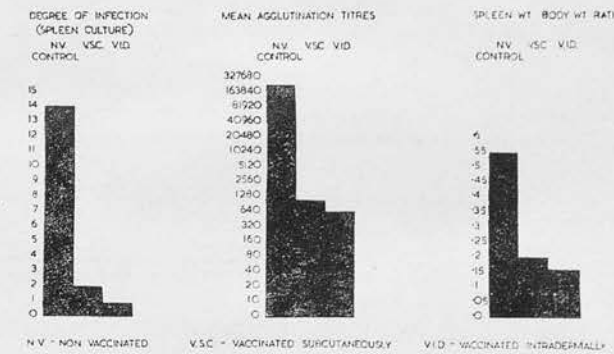

Fig. 2.

The results obtained when the guinea-pigs were killed were similar in both vaccinated groups. The control groups showed that the infective dose was adequate since 14 out of 15 animals showed infection of the spleen, the mean agglutination titre exceeded 1 in 163,840 and the spleen weight/body weight ratio was high, *viz.*, 0·55.

Little difference in the degree of infection was observed between the two vaccinated groups although the intradermal group showed a slight advantage over the subcutaneous group in all three methods used for assessing immunity.

From these results, it is suggested that the intradermal method of vaccination, if not superior to the subcutaneous route, at least gives an equally adequate protection against a test dose of 3,000,000 virulent *Br. abortus* organisms even when the amount of antigen is 25 times less than that administered subcutaneously.

Conclusions

The immunity to *Br. abortus* produced in guinea-pigs by intradermal vaccination with 0·04 ml. of S.19 vaccine compares favourably with that produced by 1 ml. of the same vaccine inoculated subcutaneously.

Acknowledgment.—I am indebted to Dr. W. S. Gordon, Director of this Field Station, for his continued interest in this work

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A Comparison of the Immunising Value in Cattle of Dead Antigens and S. 19 Br. abortus Vaccine

By

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Previous publications from this Institute (Edwards, de Ropp and McLeod, 1945 and Edwards, McDiarmid de Ropp and McLeod, 1946), describe a technique by which the immunising value of *Br. abortus* antigens can be satisfactorily measured in cattle. The vaccines tested, so far, have consisted of living organisms of reduced virulence, namely strain 45/20 (McEwen, 1937) and strain 19 (Buck, 1930). Vaccines of this type have given good protection in the immunisation of cattle in the field against brucellosis and the one now in general use in this country is that prepared from strain 19.

The desirability of using a lifeless antigen is well recognised and many attempts have been made by workers both in this and other countries to produce such a vaccine. Encouraging results were reported to the Agricultural Research Council's Committee on brucellosis by Mr. S. J. Gilbert concerning the immunisation of guinea-pigs, goats and cattle, with large doses of a vaccine composed of virulent organisms killed by formalin and suspended in lanolin and liquid paraffin. (Gilbert, 1943.)

Paterson, Pirie & Stableforth (1947), also obtained encouraging results in immunising guinea-pigs with an antigenic substance prepared from suspensions of *Br. abortus* and Paterson & Pirie (1948), indicated that this substance might be of value in the immunisation of cattle.

In view of the above findings it was decided to compare the immunising value of these dead vaccines in heifers with that of the living avirulent vaccine prepared from strain 19 and evidence will be presented to show that none of the dead vaccines equalled the efficiency of S.19.

Materials and Methods

ANIMALS

These consisted of 60 maiden Ayrshire heifers one year ten months to two years six months in age, reared in a *Br. abortus* free herd at this Field Station and negative to the agglutination test on three occasions prior to the commencement of the experiment.

They were divided into five groups and housed in isolation units (A, B, C, E and F) as described by Edwards *et al.* (1946).

VACCINATION

On December 6th, 1945 the animals were injected as follows:—

Unit A.—Twelve animals. This group was not vaccinated and was used to control the infectivity of the test dose.

Unit B.—Twelve animals, vaccinated subcutaneously at the side of the neck with 5·0 ml. of S.19 vaccine. This antigen consisted of approximately 60,000 million viable organisms and was kindly supplied by the Ministry of Agriculture's Veterinary Laboratory, Weybridge.

Unit C.—Twelve animals, vaccinated intradermally at the side of the neck with 3 mg. of an antigenic fraction of *Br. abortus* contained in 0·2 ml. of fluid. This amount of antigen would approximate the theoretical yield from 60,000 million *Br. abortus* strain 544 killed by autoclaving. (Paterson *et al.* 1947.)

Unit E.—Twelve animals, vaccinated intramuscularly in the gluteal region with 5·0 ml. of a suspension of dead *Br. abortus*. This antigen was prepared from an aerobic strain of 544 and consisted of approximately 600,000 million organisms killed by formalin and suspended in lanolin and liquid paraffin.

Unit F.—Twelve animals vaccinated in the same manner as those in Unit E except that the number of organisms employed was about 60,000 million and the inoculum was 3·0 ml.

It will be noted that apart from unit E, the antigens used were prepared from the same number of bacillary bodies, namely 60,000 million, which corresponds to the approximate number contained in one dose of strain 19 vaccine.

MATING

Three bulls were used and services commenced at the first oestrus after vaccination. One bull was used for units C, E and F, one for A and the third for B.

Owing to the unfavourable time of year when mating was commenced, some difficulty was experienced in getting the animals in calf, but 43 of the heifers were considered to have become pregnant during December and January and eight during February and March, 1946.

EXPOSURE TO VIRULENT INFECTION

On May 31st, 1946, when the majority of the heifers were about five months pregnant, virulent *Br. abortus* organisms, strain 544 (McEwen) were instilled into the conjunctival sac of each animal. The method of preparing this infective dose already has been described, Edwards *et al.* (1946) and in the present experiment the mean viable count of the dose (0·1 ml.) as determined by three separate workers was 130 million organisms. The virulence of the strain was confirmed in guinea-pigs as shown in Table I by a method similar to that described by de Ropp (1945).

TABLE I: VIRULENCE TEST OF INFECTIVE DOSE
10 GUINEA PIGS INOCULATED WITH 3 × 10^8 *Br. abortus* STRAIN 544

16 days			9 weeks		
Agglutination titre	Spleen wt./ body wt. ratio	Approx. number of *Br. abortus* in spleen	Agglutination titre	Spleen wt./ body wt. ratio	Approx. number of *Br. abortus* in spleen
640	0·19	375,000	2,560	0·34	2,400
1,280	0·27	936,000	10,240	1·34	7,800
1·280	0·19	219,000	10,240	0·97	6,600
'640	0·14	84,000	10,240	0·94	6,000
320	0·17	666,000	2,560	0·53	3,000

AGGLUTINATION TESTS

Three pre-vaccination blood samples were collected at weekly intervals from each animal and after vaccination blood was withdrawn every week for four weeks. Thereafter throughout the course of the experiment, samples were taken at intervals of one month except for an extra sample taken two weeks after the infective dose was applied.

The technique used was that described by Stableforth (1936) and the titre recorded was the highest dilution of serum producing visible agglutination.

EXAMINATION AT PARTURITION

At parturition the following materials were collected by methods already described in a previous paper, Edwards *et al.* (1946).

(*a*) blood.
(*b*) cotyledon
(*c*) colostrum
(*d*) foetal stomach contents.

All the samples from the vaccinated animals were examined culturally and biologically whereas those from the unvaccinated controls were examined biologically only if the original cultures proved negative.

CULTURAL AND BIOLOGICAL TECHNIQUE

This closely resembled the technique previously practised, Edwards *et al.* (1946), and consisted of inoculating the material on blood bacto-tryptose agar plates which were then incubated in an atmosphere containing 10 per cent. CO_2. A slight modification of the original technique consisted of adding a small quantity

of calcium chloride to the jars containing the plates, West & Borman (1945), to prevent undue moisture formation.

Five guinea-pigs were used for each sample from the vaccinated animals and two for each sample from the controls. The inoculum consisted of 1·0 ml. of the material to be examined injected intramuscularly. All the guinea-pigs were killed 6 weeks after inoculation and the presence or absence of infection was determined by the agglutination test, spleen weight/body weight ratio and spleen culture.

EXAMINATION OF *post-partum* MILK SAMPLES

Each week for the first ten weeks after parturition milk was examined from each animal for *Br. abortus*. Samples from vaccinated animals were tested by inoculation on culture medium and also biologically, whereas samples from the controls were injected into guinea-pigs only if no cultures were obtained. Two guinea-pigs were used for the biological test and the inoculum consisted of 1·0 ml. gravity cream injected intramuscularly.

Results

VACCINATION

After vaccination, all the animals in the S.19 group (Unit B) with the exception of B.38 and B.39 showed large oedematous swellings at the inoculation sites. In the group vaccinated with the Brucella "fraction" (Unit C) small hard localised swellings occurred in the majority of the animals, although the swelling in C.22 was larger and more oedematous than the rest and "slight breaking" occurred at the site of inoculation in C.28. In the two groups (Units E and F) which received lanolin vaccine no visible reactions were observed apart from slight swelling in the gluteal region.

FERTILITY RATE

Of the 60 heifers originally selected for this experiment, 51 animals eventually became pregnant. Their distribution was as follows:

Unit	A	Controls (Non-vaccinated)	11
„	B	S.19	11
„	C	"Fraction" vaccine ...	12
„	E	Dead vaccine (large dose)	9
„	F	„ „ (small dose)	8

Three animals, namely C23, C60 and B33, aborted prior to the administration of the infective dose. This was not due to brucellosis since *Br. abortus* was not recovered culturally or biologically at parturition and therefore these animals were discarded from the experiment.

UNIT A. NON-VACCINATED CONTROL GROUP

The history of each animal is shown in Table II.

Agglutination Titres.—In the control group, maximum titres of 1:80 to 1:5120 (mean 1:1149) were reached in four to 28 weeks after infection.

Infection Rate.—Of the eleven animals all, except one, subsequently developed infection. The animal which did not become infected had shown only a slight rise of titre after the infective dose was applied and her gestation period and parturition were normal. This occurred despite the fact that heavy infection was prevalent in this unit. It is possible therefore, that this animal possessed some degree of natural immunity to brucellosis.

Br. abortus was recovered from the cotyledons of ten out of eleven cows; from the colostrum of six and from the contents of the foetal stomach in five out of six dead calves.

History of Pregnancy.—The mean duration of pregnancy was 248 days; of the eleven calves six were born dead.

Infection rate in the milk.—*Br. abortus* was recovered from 37 of 110 milk samples examined, (34 per cent).

UNIT B. S.19 GROUP (60,000 MILLION ORGANISMS)

The history of this group is shown in Table II.

Agglutination Titres.—After vaccination and prior to infection maximum titres ranging from 1:320 to 1:10,240 (mean 1:3,616 developed in about two to three weeks.

Infection Rate.—Of the ten animals only two, B38 and B39, developed infection. It is interesting to note that these two animals failed to develop any local reactions when vaccinated. A CO_2 sensitive strain of *Br. abortus* was recovered from the cotyledons and colostrum of both these animals.

History of Pregnancy.—The mean duration of pregnancy of the S19 group was 274 days; eight of the calves were living and two were born dead. One of the dead calves was born from a non-infected animal, severe dystokia being the immediate cause of death, the other was due to infection with *Br. abortus*.

Infection rate in the milk.—*Br. abortus* was recovered from only two milk samples of a total of 100 examined. Both these infected samples were obtained from one of the animals that failed to resist the infective dose and both strains were CO_2 sensitive.

UNIT C. "FRACTION" VACCINE GROUP (FROM 60,000 MILLION *Br. abortus*)

The history of this group is shown in Table III.

Agglutination Titres.—After vaccination and prior to infection maximum titres ranging from 1:320 to 1:5,120 were produced in one to two weeks with a mean maximum titre of 1:1,536.

Infection Rate.—All the animals in this group became infected and the causal organism was recovered from the cotyledons of every animal, the foetal stomach of four out of five dead calves and the colostrum of seven out of nine animals sampled.

History of Pregnancy.—The mean gestation period of this group was 238 days. Five calves lived and five were born dead.

Infection Rate in the Milk.—*Br. abortus* was recovered from 42 of the 80 milk samples examined (53 per cent). One animal, C28, secreted no milk and C59 was also not sampled.

UNIT E. LANOLIN VACCINE (600,000 MILLION *Br. abortus*)

The history of this group is shown in Table III. Towards the termination of the experiment, three animals, E2, eight and nine were slaughtered for bacteriological examination by the Ministry of Agriculture's Veterinary Laboratory, Weybridge, and therefore their histories are incomplete.

Agglutination Titres.—After vaccination maximum titres ranging from 1:1,280 to 1:10,240 (mean 1:3,840) were obtained in two to three weeks.

Infection Rate.—Seven of the nine animals in this group became infected with *Br. abortus*. The organism was recovered from the cotyledons of all seven, from the foetal stomach of three out of three dead calves and from the colostrum of four out of nine samples taken. In one animal, namely E57, the infection was apparently very slight as only one guinea-pig became infected out of five injected with the cotyledon sample. Moreover, the colostrum was negative in this particular animal.

History of Pregnancy.—The mean gestation period in this group was 265 days; three dead calves were born and six lived.

Infection Rate in the Milk.—Of a total of 90 milk samples examined, ten proved positive, (11 per cent.).

UNIT F. LANOLIN VACCINE (60,000 MILLION *Br. abortus*)

The history of this group is shown in Table III. One animal, namely F11, was slaughtered ten days after calving because of symptoms of severe pyometra. A disintegrating second foetus was found at autopsy. Thus only one post-partum milk sample was obtained from this animal.

Agglutination Titres.—Table III shows that subsequent to vaccination the maximum titres reached in this group varied from 1:640 to 1:5,120 (mean 1:2,800).

Infection Rate.—Seven of the eight heifers became infected with *Br. abortus*. The organism was recovered from the cotyledons of five of the eight animals, from the foetal stomach of two out of three dead calves and from seven out of eight colostrum samples.

History of Pregnancy.—The mean gestation period was 253 days; three dead calves were born and five lived.

Infection Rate in the Milk.—*Br. abortus* was recovered from 15 out of 70 samples examined (21 per cent.).

A summary of the principal findings is shown in Table IV.

TABLE IV: SUMMARY OF RESULTS

PROTECTION AFFORDED BY DIFFERENT VACCINES AGAINST 130 MILLION *Br. abortus* STRAIN 544

Type of vaccine	Controls (Unit A)	S.19 (Unit B)	"Fraction" (Unit C)	Lanolin (Unit E)	Lanolin (Unit F)
Dose of vaccine ..	—	5·0 ml.	0·2 ml.	5·0 ml.	3·0 ml.
Route of injection ..	—	Sub-cutaneous	Intra-dermal	Intra-muscular	Intra-muscular
Heifers in groups ..	12	12	12	12	12
No. pregnant at time of test dose	11	10	10	9	8
No. of live calves ..	5	8	5	6	5
No. of heifers infected ..	10	2	10	7	7
Milk samples examined for *Br. abortus* ..	110	100	80	90	70
No. of samples positive	37	2	42	10	15

UNIT B. S.19 VACCINE

UNIT B. S.19 VACCINE

Heifer No.	Agglutination titre of serum: Weeks prior to vaccination 2	1	Weeks after vaccination 0	1	2	3	4	8	12	16	20	24	ID	Weeks after infection 2	4	8	12	16	20	24	28	32	36	History of pregnancy: Service to infection (days)	Infection to parturition (days)	Duration of pregnancy (days)	Parturition	Fate of calf	Blood serum titre	Examination at parturition: Cotyledons Cult.	Biol.	Foetal stomach Cult.	Biol.	Colostrum Cult.	Biol.
31	—	—	—	6	10	9	9	6	4	4	4	5		6	7	7	7	6	5	4	3	3		149	138	287	Dystokia	D	5	N	N 0/5	N	N 0/5	N	N 0/4
32	—	—	—	6	10	8	8	6	4	4	4	3		4	5	4	5	5	4	3	3	3		139	131	270	Nl	L	4	N	N 0/5	..	..	N	N 0/5
34	—	—	—	8	10	11	10	8	7	7	6	7		7	8	6	7	7	6	5	4	6		63	213	279	Nl	L	4	N	N 0/5	..	..	N	N 0/4
35	—	—	—	7	8	7	7	5	3	3	3	3		5	6	5	4	4	3	2	2			151	124	275	Nl	D	3	N	N 0/4	N	N 0/5	N	N 0/4
36	—	—	—	6	10	10	9	7	4	5	4	4		5	5	5	5	5	5	5	4	4		151	125	276	Nl	L	5	N	N 0/5	..	..	N	N 0/4
38	—	—	—	4	6	4	4	3	1	1	1	—		2	2	4	7	9	7	7	7	7		163	88	251	P	L	7	+	+ 5/5	..	..	+	+ 5/5
39	—	—	—	6	9	9	8	7	4	4	4	4		6	5	5	6	6	6	5	5	5		146	133	279	Nl	L	6	N	+ 5/5	..	..	N	+ 1/5
40	—	—	—	4	7	7	7	5	3	2	2	2		3	5	5	6	4	3	2	2	3		64	218	282	Nl	L	2	N	N 0/5	..	..	N	N 0/5
52	—	—	—	5	7	7	7	6	4	3	3	3		4	5	3	5	4	3	3	3	3		100	164	264	Nl	L	3	N	N 0/5	..	..	N	N 0/5
64	—	—	—	6	10	9	9	6	3	3	3	3		6	6	5	5	5	4	4	3	4		144	129	273	Nl	L	4	N	N 0/5	..	..	N	N 0/5
UNIT A. NON-VACCINATED CONTROLS														Weeks prior to infection																					
41	—	—	—	—	—	—	—	—	—	—	—	—		—	3	3	1	1	3	5	7	7		73	161	234	A	D	7	+	..	+	..	+	..
42	—	—	—	—	—	—	—	—	—	—	—	—		1	3	6	7	7	5	5	5	6		152	45	197	A	D	3	+	..	+	..	N	+ 2/2
43	1	—	1	1	—	—	—	—	—	1	1	2		2	5	6	9	9	8	7	6	7		161	71	232	A	D	7	+	..	+	..	+	..
44	—	—	—	—	—	—	—	—	—	—	—	—		2	4	5	6	7	4	4	4	2		156	88	244	P	L	6	N	+ 1/1	..	..	N	N 0/2
45	—	—	—	—	—	—	—	—	—	—	—	1		—	5	5	5	7	6	6	6	7		70	210	280	Nl	D	5	+	..	N	N 0/2	+	..
46	—	—	—	—	—	—	—	—	—	—	—	—		1	4	7	9	10	7	5	4	3		168	72	240	A	D	7	+	..	+	..	+	..
47	—	—	—	—	—	—	—	—	—	—	—	—		3	4	4	2	3	2	2	2	1		53	226	279	Nl	L	2	N	N 0/2	..	..	N	N 0/2
48	—	—	—	—	—	—	—	—	—	—	—	—		—	3	3	1	4	3	3	3	—		152	98	250	P	L	1	+	..	..	..	N	N 0/2
49	—	—	—	—	—	—	—	—	—	—	—	—		—	3	4	1	6	6	6	5	4		155	117	272	P	L	6	+	..	..	..	+	..
50	—	—	—	—	—	—	—	—	—	—	—	—		1	3	3	2	2	8	7	5	4		140	122	262	P	L	2	+	..	..	..	N	N 0/2
54	—	—	—	—	—	—	—	—	—	—	—	—		—	7	7	7	7	6	6	5	5		163	75	238	A	D	7	+	..	+	..	N	N 0/2

TABLE III

UNIT C. FRACTION VACCINE

Heifer No.	Agglutination titre: Weeks prior to vaccination 2	1	Weeks after vaccination 0	1	2	3	4	8	12	16	20	24	ID	Weeks after infection 2	4	8	12	16	20	24	28	32	36	History of pregnancy: Service to infection (days)	Infection to parturition (days)	Duration of pregnancy (days)	Parturition	Fate of calf	Blood serum titre	Examination at parturition: Cotyledons Cult.	Biol.	Foetal stomach Cult.	Biol.	Colostrum Cult.	Biol.
21	—	—	—	7	7	6	6	2	1	1	1	1		1	2	6	5	8	6	6	6	8		166	74	240	A	D	8	+	+ 4/4	+	+ 5/5	+	+ 5/5
22	—	—	—	9	9	7	7	3	1	1	1	2		3	5	8	8	7	6	6	6	7		171	47	218	A	D	7	+	+ 5/5	+	+ 5/5	+	+ 5/5
24	—	—	—	7	7	6	5	3	1	1	1	1		1	4	5	9	10	10	8	7	10		130	91	221	A	D	9	+	+ 4/5	N	+ 5/5	N	+ 5/5
25	—	—	—	6	7	7	5	3	1	1	1	2		2	1	5	6	7	6	6	5	4		170	83	253	P	L	6	+	+ 5/5	..	..	N	N 0/5
26	—	—	—	7	10	7	6	3	1	1	1	1		2	2	4	4	9	5	5	5	8		164	116	280	Nl	L	9	+	+ 5/5	..	..	N	+ 5/5
27	—	—	—	8	8	7	6	3	2	1	1	2		3	3	3	3	5	5	5	4	4		151	119	270	P	L	5	+	+ 4/4	..	..	N	N 0/4
28	—	—	—	9	8	7	5	3	1	1	1	1		2	3	7	7	6	6	6	5	4		102	53	155	A	D	7	N	+ 4/5	N	N 0/5	..	..
29	—	—	—	6	8	5	5	2	1	1	1	1		2	3	4	6	7	6	6	6	8		168	82	250	A	D	6	+	+ 5/5	N	+ 5/5	+	+ 3/3
30	—	—	—	6	6	5	5	2	1	1	1	—		—	3	6	8	10	6	6	6	10		155	88	243	P	L	10	C	+ 3/3	..	..	C	+ 5/5
59	—	—	—	5	6	4	3	2	1	—	—	—		—	1	2	4	4	4	4	4	9		141	111	252	P	L	4	+	+ 5/5	..	..	+	+ 5/5
UNIT E. DEAD VACCINE, LARGE DOSE																																			
2	—	—	—	8	9	9	8	6	5	5	5	5		5	7	6	6	7	8	8	6	..		161	108	269	P	L	7	+	+ 2/2	..	..	N	+ 1/5
4	—	—	—	7	10	10	8	8	7	7	6	6		7	7	7	7	8	9	7	7	6		157	90	247	P	L	7	+	+ 4/5	..	..	N	N 0/5
6	—	—	—	7	9	8	7	5	3	3	3	3		4	5	7	8	8	9	9	10	10		165	113	278	Nl	D	10	+	+ 4/4	+	+ 5/5	+	+ 5/5
7	—	—	—	7	9	9	8	7	6	6	5	5		6	7	6	7	7	8	8	7	7		147	115	262	P	L	7	+	+ 5/5	..	..	N	N 0/5
8	—	—	—	7	9	8	8	6	5	5	5	5		6	5	5	7	9	9	9	10	..		168	82	250	A	D	8	+	+ 5/5	+	+ 5/5	N	+ 4/5
9	—	—	—	5	10	10	9	7	6	6	6	6		7	7	6	7	8	7	7	6	..		152	80	240	A	D	7	+	+ 5/5	+	+ 5/5	+	+ 5/5
10	—	—	—	7	8	7	7	6	5	5	5	5		5	6	5	5	5	5	5	4	4		80	201	281	Nl	L	4	C	N 0/5	..	..	N	N 0/5
57	—	—	—	5	9	9	9	9	9	9	7	7		7	8	6	7	7	6	6	6	6		156	126	282	Nl	L	6	N	+ 1/5	..	..	N	N 0/5
58	—	—	1	8	10	11	11	9	8	8	7	8		8	8	7	9	8	6	6	5	6		168	108	276	Nl	L	8	N	N 0/5	..	..	N	N 0/4
UNIT F. DEAD VACCINE, SMALL DOSE																																			
11	—	—	—	5	10	9	7	5	3	4	4	5		7	7	8	9	10	..	(destroyed)				145	116	261	P	L	10	+	+ 4/4	..	..	+	+ 5/5
12	—	—	—	6	9	10	9	7	5	4	4	5		6	7	7	9	10	7	7	6	6		164	36	200	A	D	7	N	N 0/5	N	N 0/5	N	+ 5/5
14	—	—	—	7	10	10	10	7	5	5	5	5		7	8	8	9	10	10	9	8	8		169	76	245	P	L	8	+	+ 5/5	..	..	N	+ 5/5
16	—	—	—	6	9	8	8	6	5	5	5	5		5	7	7	7	7	6	5	4	4		127	153	280	Nl	L	6	N	N 0/5	..	..	N	N 0/5
17	—	—	—	5	8	8	7	4	3	3	3	4		6	6	6	7	8	8	8	8	8		145	99	244	P	D	8	+	+ 5/5	+	+ 5/5	+	+ 5/5
19	—	—	—	3	7	6	6	4	3	2	2	2		4	4	4	4	6	7	7	7	5		152	116	268	P	L	6	+	+ 5/5	..	..	C	+ 2/5
20	—	—	—	6	8	7	7	5	4	3	3	3		5	6	6	7	9	7	7	7	6		169	77	246	A	D	7	+	+ 5/5	+	+ 5/5	C	+ 5/5
56	—	—	—	7	8	7	7	6	6	5	5	5		7	7	7	8	10	8	8	8	7		104	174	278	Nl	L	7	N	N 0/5	..	..	N	+ 3/3

KEY TO TABLES II AND III

ID signifies Infective Dose applied. — signifies No agglutination titre. The agglutination titres are expressed in tube numbers, the dilutions of serum being 1/10, 1/20, 1/40, etc.
A signifies Abortion. P signifies Premature. Nl signifies Normal. D signifies Dead. L. signifies Living .. signifies Not done. + signifies Culture of *Br. abortus* obtained. N signifies Negative.
+ 4/4 signifies 4 guinea pigs infected out of 4 injected. C signifies Contaminated culture.

Discussion

One dose of S.19 vaccine as issued by the Ministry of Agriculture's laboratory at Weybridge contains approximately 60,000 million organisms and two of the dead antigens used in this experiment were prepared from approximately the same number of bacillary bodies. The third dead vaccine contained ten times this number, namely 600,000 million. Details of Gilbert's work subsequent to that reported in his original paper (1943) have not been published. Reference was, however, made to it in a general review (Stableforth, 1947), from which it is clear that, although the larger vaccine dose used in the work reported here did not protect adequately against the test dose in these experiments (130 million) it gave a high degree of protection against a test dose of 15 million, *i.e.*, a test dose which produces infection in most non-vaccinated animals (cf. Gilbert's controls *loc. cit.* and Edwards *et al.*, 1946) and one that is probably higher than that acquired by animals under conditions of natural infection. Arising from the work described in this paper, it will be observed that the living avirulent S.19 vaccine is preferable to the dead antigens even when the number of virulent organisms used to prepare the dead vaccine is increased tenfold. It is probable that a better degree of immunity might be produced by large and repeated doses of one of these dead antigens, especially the lanolin vaccine, which, in the dosage used in this experiment, appeared to lessen the severity of the ensuing experimental infection. The disadvantages associated with large and repeated dosage, would, however, in the field, far outweigh the advantages associated with a lifeless antigen. As a result of this and previous experiments, there is little doubt that S.19 vaccine remains the most reliable antigen for immunising cattle against brucellosis.

Conclusion

An antigenic substance prepared from killed *Br. abortus* and dead vaccines consisting of whole organisms suspended in an oily base, failed to equal the efficiency of the living avirulent strain 19 in immunising cattle against brucellosis.

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The Stability of the Avirulent Characters of Brucella Abortus, Strain 19 and Strain 45/20 in Lactating and Pregnant Cows

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McEwen (1940) demonstrated the instability of the avirulent characters of *Br. abortus* strain 45/20 in pregnant cattle. Subsequently, Edwards, de Ropp and McLeod (1945), during the course of their immunological experiments with 45/20, could not exclude the possibility that even in non-pregnant cattle it might alter in character and regain its former virulence. On the other hand, McEwen (1946) found no evidence that this strain became virulent when used for vaccinating non-pregnant cattle in the field. The stability of the avirulent characters of *Br. abortus* strain 19 has been confirmed by several American workers (Mingle, Manthei & Jasmin, 1941; Birch, Gilman & Stone, 1943; and Gerhard & Wilson, 1948), and in this country by Edwards, McDiarmid, de Ropp & McLeod (1946), who showed that this strain is harmless when injected into non-pregnant heifers and that the organism is not excreted in the milk during the subsequent lactation. Moreover, McDiarmid (1949) has shown that, in certain circumstances, pregnant cattle may be vaccinated with strain 19 with comparative safety.

The object of the work described in this paper was to study further the stability in cattle of both strains 19 and 45/20. Part I refers to the inoculation of lactating cows with vaccinal doses, whereas Part II describes the passage of each strain in large intravenous dosage through pregnant cattle.

PART I

MATERIAL AND METHODS

In general, the methods employed were similar to those described by Edwards, McDiarmid, de Ropp and McLeod (1946) and may briefly be described as follows:—

Experimental Animals.—Twenty-eight non-pregnant Ayrshire and Friesian cows negative to the agglutination test. These animals had already calved once and, with three exceptions, were lactating at the time of vaccination.

Vaccination.—The routine method of subcutaneous injection was used. Eight cows received 5·0 ml. of S.19 vaccine; eight received two separate inoculations of 4·0 ml. 45/20 vaccine at an interval of three weeks. The vaccines were obtained from the Ministry of Agriculture.

Agglutination Tests.—The scheme of Stableforth (1936) was used.

COLLECTION AND EXAMINATION OF MATERIAL

This has already been described by Edwards *et al.* (1946). Duplicate plates of bactotryptose blood agar were inoculated with samples of colostrum, cotyledon or milk and incubated aerobically and in 10 per cent. CO_2. This technique was checked by the culture of known *Br. abortus* strains under the same conditions. Guinea-pigs used for the biological tests were killed three weeks after inoculation to ensure the recovery of S.19 if this strain should be present.

EXPERIMENTAL PROCEDURE

The 28 cows were housed in the same dairy and grazed in the same fields and were divided into three groups, *viz.*:—

(1) 45/20 group—eight animals.

(2) S.19 group—eight animals.

(3) Non-vaccinated controls—twelve animals.

Services commenced two months after vaccination. Prior to parturition the following samples were taken:—

Milk samples at fortnightly intervals until the animal became dry. These were examined culturally in all cases and in addition samples from the vaccinated animals were tested biologically at monthly intervals.

Blood for the agglutination test 14 days after vaccination and thereafter at monthly intervals.

At parturition the following materials were collected:—

(1) Blood for agglutination test.

(2) Colostrum.

(3) Cotyledon or Cervical Swab.

For cultural and biological examination in the vaccinated groups and cultural examination only in the non-vaccinated group.

After parturition, in the subsequent lactation, milk samples from each cow were examined culturally every 14 days for a period of ten weeks and milk from the vaccinated animals was examined biologically at four and eight weeks. In addition blood samples for agglutination tests were taken from each animal at intervals of one month after calving.

Results

The results are shown in Table I and it will be seen that no evidence was obtained of the excretion of *Br. abortus* from lactating cattle vaccinated with strain 19 or 45/20. Moreover, no infection was demonstrated in the in-contact control animals.

TABLE I: THE BACTERIOLOGICAL EXAMINATION OF CATTLE VACCINATED WITH STRAINS 19 AND 45/20 AND NON-VACCINATED CONTROLS

Group	Pre partum milk samples		Maximum mean agglutination titre subsequent to vaccination and prior to parturition	Examination at parturition					Post partum milk samples	
				Mean blood titre	Colostrum		Cotyledon			
	Cult.	Biol.			Cult.	Biol.	Cult.	Biol.	Cult.	Biol.
45/20 (8 cows) ..	105 N	32 N	N	N	8 N	8 N	8 N	8 N	40 N	16 N
S.19 (8 cows*) ..	66 N	29 N	1 : 3,400	1 : 240	6 N	6 N	6 N	6 N	30 N	12 N
Controls (12 cows) ..	176 N	..	N	N	12 N	12 N	12 N	12 N	101 N	..

N signifies negative. .. signifies not done. * signifies one cow destroyed prior to parturition and one cow not pregnant.

PART II

Experimental Procedure

The scheme of the experiment was to inoculate each strain into a pregnant cow in a dose sufficient to produce abortion, recover the organisms in culture, then with as short a period as possible on artificial medium inoculate another pregnant animal. This procedure was repeated until both strains had been passaged seven times. After each passage an attempt was made to assess, both *in vitro* and in guinea-pigs, any departure from the recognised characteristics of the strains.

Materials and Methods

Cultures.—Both strains were received in the dry state from the Veterinary Laboratory, Weybridge, subcultivated on bactotryptose agar and a 48-hour subculture from this was used to inoculate the first passage animals. At abortion, cotyledon and/or foetal stomach contents were spread over blood-bactotryptose agar plates, incubated in duplicate in air and 10 per cent. CO_2 and, when possible, 50 colonies picked off. All of these were tested by the thionin blue method (McLeod, 1944) for the identification of S.19, then one half was used in phase determination by the thermo-agglutination method, and the other subcultivated and mixed to provide inocula for the next passage and for virulence determination in guinea-pigs. Thus the organisms grew *in vitro* twice between each passage.

Experimental Animals.—Fourteen cattle, four to six months in calf, from a herd consistently negative to the agglutination test, were used. Before and after inoculation, precautions were taken to avoid all risk of extraneous infection. Each animal was inoculated intravenously with approximately 20 times the recommended vaccine dose of S.19 in a volume of 50 ml. The mean viable counts were: S.19, 14×10^{11} organisms, and 45/20, 11×10^{11} organisms.

Assessment of Virulence in Guinea-pigs

It is known that strain 19, after inoculation in suitable dosage into guinea-pigs, produces neither splenic enlargement nor persistent agglutinins and may be recovered culturally from the spleen three weeks but not seven weeks after inoculation. Similarly, strain 45/20 produces neither agglutinins nor splenic enlargement and may be recovered seven weeks but not 15 weeks after inoculation. A virulent strain, on the other hand, causes persistent agglutinins in high titre, marked splenic enlargement and may be recovered in culture from the spleen up to six months after inoculation.

Accordingly, groups of 15 guinea-pigs were inoculated subcutaneously with each strain, *i.e.*, prior to passage and after recovery from each cow. The infecting dose in each case was calculated as one million organisms (average viable count: S.19, 85×10^4 organisms; 45/20, 77×10^4 organisms). Five guinea-pigs from each group were destroyed three, seven and 15 weeks after inoculation and the agglutination titre, spleen weight/body weight ratio (de Ropp, 1945) and the presence or absence of Brucella in the spleen determined.

Results

Table II shows the result obtained when *Br. abortus* S.19 is passaged in large intravenous dosage seven times through pregnant cattle. In so far as was determined the strain remained unchanged; it retained both its ability to grow in air and its accepted virulence for guinea-pigs; its phase remained smooth. Further, all of the 350 or so single colonies examined by the thionin blue test exhibited the specific reaction shown to this dye by S.19.

Strain 45/20, on the other hand (Table III), by the seventh passage had become a highly virulent CO_2 sensitive strain. Its "R" character, and consequently its inability to produce agglutinins, was lost between the second and third passage and the inoculation of further animals merely served to emphasise and perhaps to enhance, its altered virulence. Sensitivity to CO_2 returned more gradually. This was first observed after the third passage, when the organisms recovered showed a greater colonial size when incubated in CO_2 than in air. By the seventh passage, organisms incubated for 48 hours in 10 per cent. CO_2 were of normal size, whereas after ten days' incubation in air they were just visible to the naked eye.

Summary

1. Neither *Br. abortus* strain 19 nor strain 45/20 was recovered from the cystic products of cattle vaccinated during lactation.

2. *Br. abortus* strain 19 retained its cultural and biological characteristics after seven serial passages in large intravenous dosage through pregnant cattle, while strain 45/20 on similar passage reverted to a fully virulent CO_2 sensitive strain.

Acknowledgment

We are indebted to the Director of the Field Station, Dr. W. S. Gordon, for his continued interest in this work.

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Table II: The Passage of *Brucella abortus* Strain 19 Through Pregnant Cattle

Passage	Abortion		Phase		Virulence for guinea pigs								
					3 weeks			7 weeks			15 weeks		
	Days	Titre	"S"	"R"	Ratio	Titre	*Br. ab.*	Ratio	Titre	*Br. ab.*	Ratio	Titre	*Br. ab.*
			25	0	0·14	1 : 40	+	0·14	1 : 20	—	0·16	N	—
1	16	1 : 1,280	25	0	0·12	1 : 76	+	0·12	1 : 4	—	0·15	N	—
2	25	1 : 2,560	25	0	0·17	1 : 34	+	0·17	1 : 6	—	0·12	N	—
3	41	1 : 5,120	24	1	0·18	1 : 84	+	0·11	1 : 6	—	0·13	N	—
4	42	1 : 320	25	0	0·17	1 : 24	+	0·11	N	—	0·12	N	—
5	32	1 : 2,560	25	0	0·13	1 : 38	+	0·13	1 : 4	—	0·11	N	—
6	30	1 : 5,120	25	0	0·12	1 : 52	+	0·13	1 : 10	—	0·12	N	—
7	24	1 : 20,480	25	0	0·11	1 : 18	+	0·15	1 : 6	—	0·12	N	—

N signifies negative in a dilution of 1 : 10.

Table III: The Passage of *Brucella abortus* Strain 45/20 Through Pregnant Cattle

Passage	Abortion		Phase		Virulence for guinea pigs								
					3 weeks			7 weeks			15 weeks		
	Days	Titre	"S"	"R"	Ratio	Titre	*Br. ab.*	Ratio	Titre	*Br. ab.*	Ratio	Titre	*Br. ab.*
			0	25	0·12	N	+	0·15	N	+	0·13	N	—
1	12	1 : 20	0	25	0·12	N	+	0·16	N	+	0·13	N	—
2	37	1 : 20	0	10	0·12	N	+	0·15	N	+	0·15	N	—
3	47	1 : 20	10	15	0·14	1 : 128	+	0·28	1 : 1,696	+	0·53	1 : 10,240	+
4	37	1 : 2,560	25	0	0·16	1 : 256	+	0·41	1 : 6,672	+	0·65	1 : 74,242	+
5	24	1 : 10,240	25	0	0·13	1 : 432	+	0·49	1 : 960	+	0·47	1 : 14,366	+
6	10	1 : 1,280	25	0	0·17	1 : 352	+	0·51	1 : 3,840	+	0·56	1 : 19,200	+
7	9	1 : 2,560	25	0	0·16	1 : 352	+	0·56	1 : 4,416	+	0·37	1 : 13,312	+

N signifies negative in a dilution of 1 : 10.

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A COMPARISON OF THE IMMUNITY PRODUCED IN CATTLE BY THE INOCULATION OF *BR. ABORTUS* STRAIN 19 INTRADERMALLY, INTRACAUDALLY AND SUBCUTANEOUSLY

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Previous experiments carried out in this country have shown that S.19 *Br. abortus* vaccine is a reliable and effective antigen for immunising cattle against contagious abortion (Edwards, McDiarmid, de Ropp & McLeod (1946), McDiarmid (1949 a and b) and Taylor & McDiarmid (1949)). As the demand for this vaccine will increase and in view of the difficulties of production, it is essential to investigate any method of administration that might diminish the dose required without impairing its antigenic power.

Campbell and Rodwell (1945) reported that the intradermal injection of 0·2 ml. of S.19 vaccine produced high agglutination titres in cattle similar to those obtained by 25 times this dose given subcutaneously, and that still higher titres followed the intracaudal injection of 1 ml. They deduced from these experiments that high titres indicated a satisfactory degree of immunity, but this supposition was not confirmed by exposure of the cattle to a known experimental infection. To confirm the work of Campbell and Rodwell, McDiarmid (1948) carried out a preliminary experiment in guinea-pigs and found that an intradermal dose of 0·04 ml. of S.19 vaccine produced as good an immunity as 25 times this dose given subcutaneously. The present paper concerns similar experimental work in cattle and it will be shown that small doses either intradermally or intracaudally produce a high degree of immunity.

MATERIALS AND METHODS

Animals.—These consisted of 42 Ayrshire heifers 15 to 18 months of age reared in a *Br. abortus* free herd at this field station and negative to the agglutination test on two occasions prior to the commencement of the experiment. They were divided into four groups and housed in isolation units.

Vaccination.—S.19 *Br. abortus* vaccine, as issued from the Ministry of Agriculture's Veterinary Laboratory at Weybridge, was inoculated as follows:—

(1) Subcutaneous group (ten heifers).—A dose of 5·0 ml. was injected under the skin in the chest wall just behind the olecranon process. After vaccination the area was massaged.

(2) Intradermal group (ten heifers).—The same area was chosen and 0·2 ml. was injected by the technique ordinarily employed for tuberculin testing.

(3) Intracaudal group (ten heifers).—1·0 ml. was inoculated into the fibrous tissue under the skin about half an inch from the tip of the tail.

(4) The remaining 12 animals constituted the non-vaccinated control group.

Local effect of vaccination

(1) Subcutaneous group.—When the animals were examined one week after vaccination the usual large oedematous swellings were present. They measured up to 10 inches in diameter in some instances, and were sensitive to touch, but no lameness was noticed. Two weeks after vaccination the large swellings had subsided and only a slight hardening of the underlying tissues could be palpated.

(2) Intradermal group.—One week after vaccination the majority of the animals showed a reaction area about three centimetres in diameter with a small necrotic spot in the centre. The necrotic area had commenced to slough in some animals, but no sensitivity was noticed. Fourteen days after vaccination all the animals showed only slight scabs at the inoculation sites.

(3) Intracaudal group.—One week after vaccination, apart from some cylindrical swelling involving the whole of the tip of the tail, no abnormality was noticed; the tails did not appear to be unduly sensitive and seven days later the tails appeared normal.

Mating.—Two bulls were used and mating was commenced at the first oestrus after vaccination; with two exceptions all the heifers proved to be in calf prior to the application of the infective dose.

Exposure to virulent infection.—When the majority of heifers were about five months in calf approximately 150 million virulent *Br. abortus* organisms, Strain 544, were instilled into the conjunctival sac of each animal. The virulence of the suspension of organisms was confirmed in guinea-pigs as shown in Table I.

TABLE I

VIRULENCE TEST OF INFECTIVE DOSE

Ten guinea-pigs inoculated with 3×10^6 Strain 544

Sixteen days			Nine weeks		
Agglutination titre	Spleen wt. per body-weight ratio	Approx. No. of *Br. abortus* in spleen	Agglutination titre	Spleen wt. per body weight ratio	Approx. No. of *Br. abortus* in spleen
320	0·14	358,000	81,920	0·43	1,200
2,560	0·30	183,000	2,560	0·24	1,500
640	0·13	397,000	163,840	0·54	5,400
1,280	0·22	378,000	2,560	0·41	1,400
1,280	0·25	333,000	Guinea-pig died—not examined		

Agglutination tests.—Blood samples from all the heifers were examined at monthly intervals throughout the course of the experiment by the technique of Stableforth (1936). Additional tests were made immediately after vaccination and infection.

Examination of materials collected after parturition.—In addition to agglutination tests, cotyledons, colostrum, foetal stomach contents and milk samples were examined culturally and biologically for the presence of *Br. abortus*

by methods similar to those employed by Edwards *et al.* The examination of milk from each heifer was carried out once each week for ten weeks after parturition.

Results

Table II shows in detail the collected data relating to each experimental animal. Table III (page 364) consists of a summary of the principal findings and shows the incidence of *Br. abortus* in the milk after parturition, and Fig. 1 (page 364) depicts the mean agglutination titres for each of the four groups throughout the course of the experiment.

Non-vaccinated control group.—The infective dose in this experiment appeared to be adequate since all 12 animals in this group became infected and ten calves were born dead. One animal, namely, J 97, appeared to have a very slight degree of infection since *Br. abortus* was detected only in the colostrum, and there by biological examination only. Numerous milk samples from this group were shown to be infected.

Subcutaneous group.—The results corresponded to those obtained in previous experiments with this particular dose; only one out of a total of eight animals became infected and six living calves were born. No infection was detected in the milk from these heifers.

Intradermal group.—In this group the immunity produced was of a high order. Nine animals were protected out of ten and nine living calves obtained. The mean titres, as shown in Fig. 1, were similar throughout the course of the experiment to those obtained in the group inoculated subcutaneously. No milk samples were infected.

Intracaudal group.—The protection rate was similar to the previous group, two animals out of ten developing in-

TABLE

COMPARISON OF IMMUNITY PRODUCED BY VARIOUS DOSES

	Heifer No.	Agglutination titres of serum: Weeks prior to vaccination		Weeks after vaccination I. Dose																		
		1	0	1	2	3	4	8	12	16	20	24	28	32	36	40	44	48	52	56	60	64
5 ml. S.C.	J 70	—	—	7	13	13	12	8	6	6	6	5	6	6	5	6	6	6	6	6	6	6
	J 75	—	—	7	10	9	7	4	3	2	3	3	3	3	3	3	6	2	2	2	2	2
	J 92	—	—	7	9	8	8	6	5	4	4	4	4	4	4	6	5	5	5	4	4	3
	J 96	—	—	5	9	9	7	6	5	4	4	...	...	...	...	...	...	...	...	...	...	NP
	J118	—	—	6	11	10	10	7	6	5	5	4	5	6	5	5	7	6	6	5	6	6
	J171	—	1	8	13	14	12	9	6	6	5	...	...	...	...	...	...	...	...	...	...	NP
	J184	—	—	6	10	10	8	5	5	4	4	3	4	4	5	6	2	4	4	4	4	5
	J185	—	—	7	9	9	9	5	4	3	3	3	5	3	3	3	2	2	2	2	2	2
	643	—	—	5	9	9	6	5	5	3	3	4	5	4	4	3	Died					
	K15	—	—	8	8	8	6	5	4	3	2	2	3	3	3	1	3	2	2	2	2	1
1 ml. I.C.	J 21	—	—	6	10	10	11	8	6	6	5	5	5	5	5	5	5	Died				
	J 72	—	1	8	12	12	10	7	5	5	4	4	5	4	4	3	4	3	3	4	4	4
	J119	—	—	10	13	12	13	8	7	7	7	6	7	8	9	15	9	10	10	10	8	11
	J127	—	1	11	14	15	11	7	6	6	6	6	7	8	4	8	7	5	5	6	6	6
	J139	—	1	9	10	10	7	6	5	6	5	5	5	4	5	5	4	4	4	5	5	4
	J140	—	—	6	9	9	9	6	6	5	5	4	5	5	4	4	6	5	5	5	5	5
	J155	—	—	8	13	12	9	7	6	4	4	4	6	6	4	7	6	6	6	5	6	6
	J187	—	—	10	12	11	9	7	5	6	6	5	6	5	5	5	6	6	6	5	5	4
	662	—	—	7	12	12	10	9	8	6	7	7	7	9	8	10	8	6	Dead			
	670	—	—	9	12	12	10	6	5	5	4	4	5	4	4	4	5	5	5	4	5	4
0·2 ml. I.D.	J 66	—	—	4	13	10	9	7	5	5	5	4	4	5	4	4	5	5	5	5	5	4
	J123	—	—	7	10	9	8	7	5	5	5	5	5	4	4	5	5	5	5	5	5	4
	J128	—	1	5	8	8	7	4	5	4	4	4	7	4	5	9	5	4	4	4	4	5
	J147	—	—	5	10	9	6	5	4	3	3	3	2	3	3	4	4	3	3	3	3	3
	J160	—	—	6	12	11	9	6	6	5	4	4	4	6	5	4	5	5	5	5	5	3
	J169	—	—	3	10	11	7	6	5	5	4	3	4	4	3	3	4	3	3	3	3	4
	J172	—	—	5	9	8	7	6	5	3	3	3	4	3	3	3	3	3	Dead			
	J177	—	—	7	11	10	8	6	5	5	5	5	5	6	5	5	7	6	6	5	5	4
	J188	—	—	4	9	9	6	5	4	3	3	3	4	4	3	3	3	3	3	4	4	3
	K 6	—	—	4	10	9	7	6	5	4	4	5	5	4	4	3	4	4	4	4	4	2
Control group	J 71	—	—	1	—	1	2	1	1	—	—	—	5	6	9	8	7	6	6	6	6	6
	J 85	—	—	—	—	1	—	—	—	—	—	—	3	3	8	13	9	8	8	9	9	12
	J 97	—	—	—	—	—	—	—	—	—	—	—	3	1	1	—	5	6	6	6	6	8
	J166	—	—	—	—	—	—	—	—	—	—	—	4	4	9	8	6	5	5	4	2	3
	J173	—	—	—	—	—	—	—	—	—	—	—	8	9	7	6	6	5	5	5	5	7
	J178	—	—	—	—	—	—	—	—	—	1	—	5	7	4	5	6	8	8	7	6	9
	J186	—	—	1	—	1	—	—	1	—	—	—	5	5	7	8	7	7	6	5	5	4
	J189	—	—	—	—	—	1	—	—	—	—	—	4	6	7	7	6	6	6	5	4	4
	K 2	—	—	—	—	1	1	1	1	—	—	—	5	8	6	6	6	6	6	6	6	6
	K 7	—	—	1	—	—	—	—	—	—	—	—	7	9	8	6	5	4	4	4	4	4
	K 8	—	—	—	—	1	—	—	—	—	—	—	6	7	6	5	6	5	5	4	4	3
	K17	—	—	—	1	1	2	1	1	1	—	—	6	6	5	2	2	2	2	2	2	2

The agglutination titres are expressed as tube numbers except at parturition.

I. dose signifies Infective dose applied
NP ,, Non-pregnant
D ,, Dead
+ ,, Positive

S.C. signifies subcutaneously
... ,, Not done
TA. ,, Typical abortion
N. ,, Negative

fection. The most interesting feature was the high titre reached in the majority of animals after vaccination as shown in Fig. 1. One milk sample was found to contain a CO_2 sensitive strain of *Br. abortus*.

Discussion

This experiment achieved the practical object of showing that smaller doses than those hitherto employed for the routine subcutaneous injection could confer an effective immunity if injected intradermally or intracaudally. Certain advantages are associated with the immunisation of cattle by 0·2 ml. intradermally. The quantity of vaccine required is diminished considerably, thereby reducing the cost of production and lessening the technical difficulties associated with the production of large quantities of viable antigen. The technique of inoculation is simplified, especially for those accustomed to routine tuberculin testing. The local reaction is slight and severe systemic disturbances are eliminated; this, we believe, might be of value in the inoculation of lactating animals with a view to avoiding a decrease in the milk yield; it has already been shown that 5·0 ml. subcutaneously has an immediate depressing effect on the secretion of milk, the loss averaging 24 lb. (Holman & McDiarmid (1945)). The intracaudal method also has the undoubted advantage of ease of administration, especially so in the case of dairy cows limited in their movements by the yoke system of tying.

Owing to the limited capacity of the isolation units it was not possible to compare the effect of the intradermal or intracaudal method against decreasing doses given subcutaneously. It could, therefore, be argued that a smaller

II

OF S.19 VACCINE ADMINISTERED BY DIFFERENT ROUTES

History of pregnancy						Tests at parturition					
						Cotyledons		Foetal stomach		Colostrum	
Service to infection (days)	Infection to parturition (days)	Duration of pregnancy (days)	Par-turition	Fate of calf	Blood serum titre	Cult.	Biol.	Cult.	Biol.	Cult.	Biol.
141	143	284	Nl.	L.	320	N.	N 0/5	...	...	N.	N 0/5
158	120	278	Nl.	L.	40	N.	N 0/5	...	...	N.	N 0/5
143	144	287	Nl.	L.	160	N.	N 0/5	...	...	N.	N 0/5
148	140	288	Nl.	L.	320	N.	N 0/5	...	...	N.	N 0/5
110	109	219	TA.	D.	320	+	+ 5/5	+	+ 5/5	N.	N 0/5
154	129	283	Nl.	L.	20	N.	N 0/5	...	...	N.	N 0/5
146	134	280	Dystokia	D.	160	N.	N 0/5	...	...	N.	N 0/5
145	135	280	Nl.	L.	40	N.	N 0/5	...	...	N.	N 0/5
114	166	280	Dystokia	D.	160	N.	N 0/3	N	N 0/5	N.	N 0/4
149	129	278	Nl.	L.	160	N.	N 0/5	...	...	N.	N 0/5
129	104	233	P.	K.	163,840	+	+ 5/5	N.	N 0/5	N.	+ 5/5
156	131	287	Nl.	L.	1,280	N.	N 0/5	...	...	N.	N 0/5
152	127	279	Nl.	L.	160	N.	N 0/5	...	...	N.	N 0/5
83	198	281	Nl.	L.	320	N.	N 0/5	...	...	N.	N 0/5
153	100	253	P.	D.	640	+	+ 5/5	N.	N 0/5	N.	N 0/5
160	121	281	Nl.	L.	160	N.	N 0/5	...	...	N.	N 0/5
148	126	274	Nl.	L.	1,280	N.	N 0/5	...	...	N.	N 0/5
98	180	278	Nl.	L.	160	N.	N 0/5	...	...	N.	N 0/5
151	131	282	Nl.	L.	160	N.	N 0/5	...	...	N.	N 0/5
153	131	284	Nl.	L.	160	N.	N 0/5	...	...	N.	N 0/5
134	107	241	TA.	D.	320	+	+ 5/5	+	+ 4/5	N.	N 0/5
136	150	286	Nl.	L.	80	N.	N 0/5	...	...	N.	N 0/5
134	148	282	Nl.	L.	80	N.	N 0/5	...	...	N.	N 0/5
128	159	287	Nl.	L.	80	N.	N 0/5	...	...	N.	N 0/5
130	152	282	Nl.	L.	40	N.	N 0/3	...	...	N.	N 0/5
145	136	281	Nl.	L.	160	N.	N 0/5	...	...	N.	N 0/5
157	130	287	Nl.	L.	40	N.	N 0/5	...	...	N.	N 0/5
145	142	287	Nl.	L.	80	N.	N 0/5	...	...	N.	N 0/5
151	60	211	TA.	D.	320	+	+ 3/3	+	+ 3/3	N.	N 0/2
153	99	252	P.	L.	5,120	+	+ 3/3	...	...	+	+ 3/3
158	123	281	Nl.	L.	40	N.	N 0/5	...	...	N.	+ 5/5
145	78	223	TA.	D.	640	+	+ 3/3	+	+ 2/2	N.	N 0/3
160	70	230	TA.	D.	2,560	+	+ 3/3	N.	+ 1/2	N.	+ 3/3
118	45	163	TA.	D.	640	+	+ 3/3	+	+ 3/3	N.	+ 1/3
137	71	208	TA.	D.	1,280	+	+ 3/3	N.	+ 2/2	N.	+ 2/3
?	61		TA.	D.	1,280	+	+ 2/2	N.	N 0/3	N.	+ 2/2
147	56	203	TA.	D.	1,280	+	+ 3/3	+	+ 2/2	N.	+ 1/3
144	47	191	TA.	D.	2,560	+	+ 3/3	+	+ 3/3	N.	+ 1/3
81	48	129	TA.	D.	320	+	+ 3/3	+	+ 3/3	N.	N 0/3
145	28	173	TA.	D.	320	+	+ 3/3	+	+ 3/3	N.	+ 1/3

I.C. signifies Intracaudally
Nl. „ Normal
P. „ Premature
N 0/5 „ Five guinea-pigs negative

I.D. signifies Intradermally
L. „ Living
K. „ Killed by cow

FIG. I

MEAN AGGLUTINATION TITRES

OF IMMUNE VACCINATED HEIFERS AND NON VACCINATED CONTROLS.

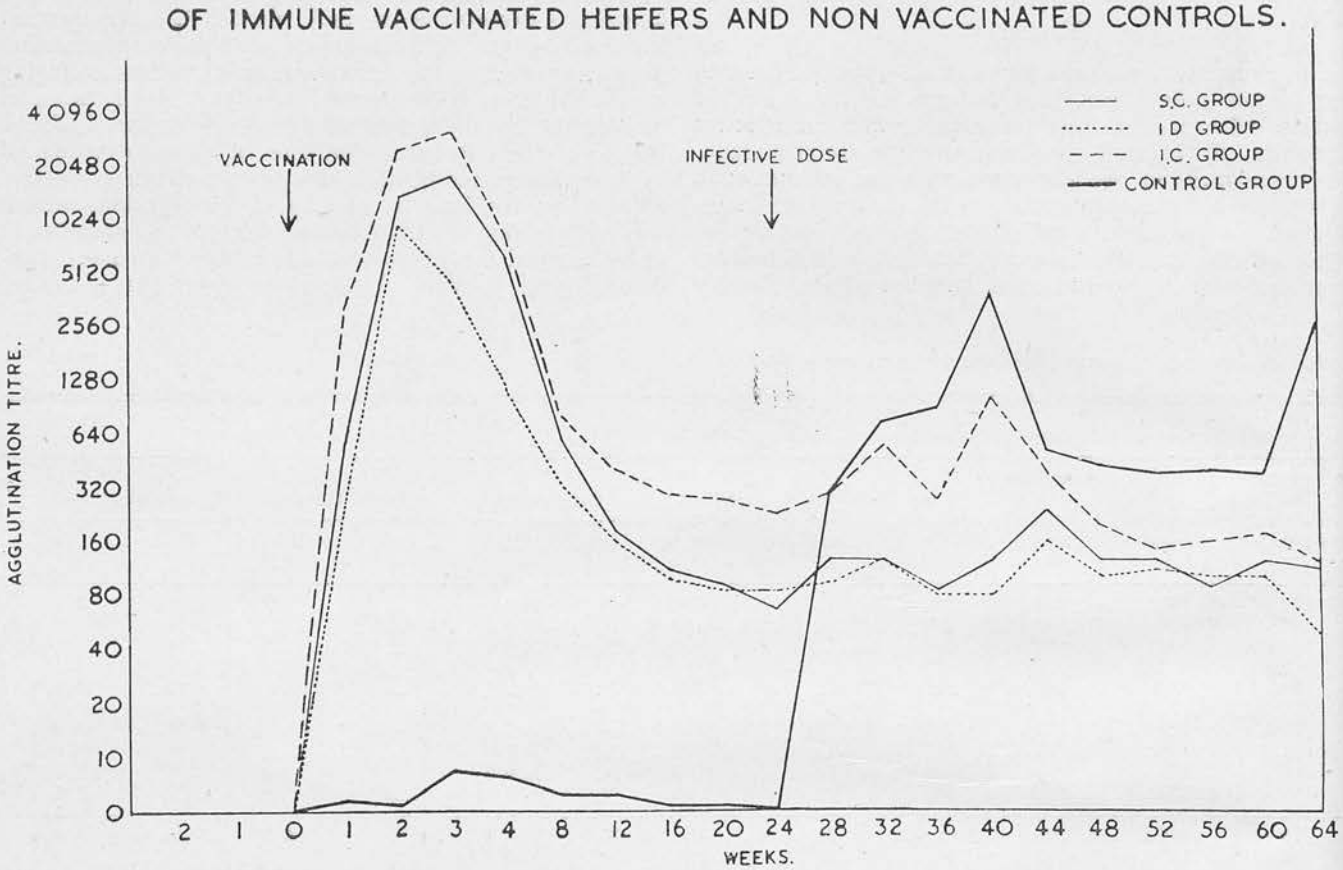

TABLE III

SUMMARY OF RESULTS

Protection by S.19 vaccine against 150 million Strain 544 *Br. abortus*

Route of injection	Subcutaneous	Intradermal	Intracaudal	Controls
Dose of vaccine	5·0 ml.	0·2 ml.	1·0 ml.	0
Heifers in groups	10	10	10	12
Number pregnant at time of test dose	8	10	10	12
Test dose of 150 million *Br. abortus* Strain 544 ...	+	+	+	+
Number of live calves ...	6	9	7	2
Number of cows infected ...	1	1	2	12
Milk samples examined culturally for *Br. abortus* ...	70	100	88	120
Number of samples positive	0	0	0	3*
Milk samples tested biologically	70	100	88	118
Number of samples positive	0	0	1	24†

* Signifies from three different cows.
† Signifies from ten different cows.

dose given by the subcutaneous route would have been as effective as the routine 5·0 ml. dose, and further work is in progress to determine the equivalent values of the antigen given by these different methods.

All these facts must be considered when assessing the relative merits of the methods under discussion. We may, however, tentatively suggest that, should the demand for vaccine become so great as to embarrass the source of supply, the intracaudal or preferably the intradermal route of administration might be considered as a possible alternative.

CONCLUSION

The intradermal inoculation of 0·2 ml. or the intracaudal inoculation of 1·0 ml. S.19 *Br. abortus* vaccine confers an immunity in heifers comparable to that produced by the subcutaneous inoculation of 5·0 ml. of the same antigen.

Acknowledgment.—I wish to express my thanks to Dr. W. S. Gordon, director of this field station, for his continued interest and advice and to Mr. S. J. Edwards for applying the infective dose. The work was carried out under the general supervision of the Agricultural Research Council's Committee on Brucellosis.

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THE OCCURRENCE OF *VIBRIO FOETUS* IN ABORTED MATERIAL DERIVED FROM COWS INOCULATED WITH S. 19 *BR. ABORTUS* VACCINE

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With the routine use of S. 19 vaccine, brucellosis has been checked to such a degree that already the disease is considered of little economic importance in some parts of the country (Clark 1949, Stewart 1950). Control of this disease, however, has made more evident the existence of other types of abortion.

With the kind co-operation of practising veterinary surgeons, an investigation of the causes of abortion in herds inoculated against *Br. abortus* has been commenced, and it will be shown in this paper that in some vases *V. foetus* is associated with the occurrence of abortion.

MATERIALS AND METHODS

Twenty herds of various breeds of cattle were available for examination in Berkshire; all had a similar history of previous vaccination with S. 19. When an abortion occurred various materials were collected and forwarded to the laboratory as soon as possible; these consisted of:—

(*a*) Foetal stomach contents (if available).
(*b*) Cotyledons (if available).
(*c*) Colostrum.
(*d*) Blood.

The amount of material collected from the different herds was as follows:—

Number of herds, 20; number of abortions, 32; number of blood samples, 29; number of cotyledon samples, 7; number of foetuses, 23; number of colostrum samples, 25.

Cotyledons were rarely received, occasionally the foetus was unobtainable, and in three instances a foetus was delivered to the laboratory without a blood or milk sample.

BACTERIOLOGICAL EXAMINATION

It was essential, in the first instance, to eliminate the possibility of *Br. abortus* infection in these animals despite the history of vaccination with S. 19, and therefore serological, cultural and biological examinations were performed according to the technique described in previous papers, *e.g.*, McDiarmid (1949). In the subsequent search for *V. foetus*, the following methods were adopted:—

(1) Smears from the foetal stomach contents and/or cotyledons were stained by dilute carbol fuchsin. These were examined for the characteristic spiral forms of the organism. (See photomicrograph, Fig. 1.)

(2) Foetal stomach contents and/or a suspension of cotyledons were streaked in 5 per cent. ox blood agar slopes and also inoculated into 0·3 per cent. soft agar medium containing 5 per cent. ox blood. These media were contained in McCartney bottles in which a suitable atmosphere for growth was created by screwing the caps down firmly; they were examined after three to six days' incubation at 37° C. The presence of *V. foetus* was confirmed by microscopic examination, and a typical smear from a culture is shown in photomicrograph, Fig. 2.

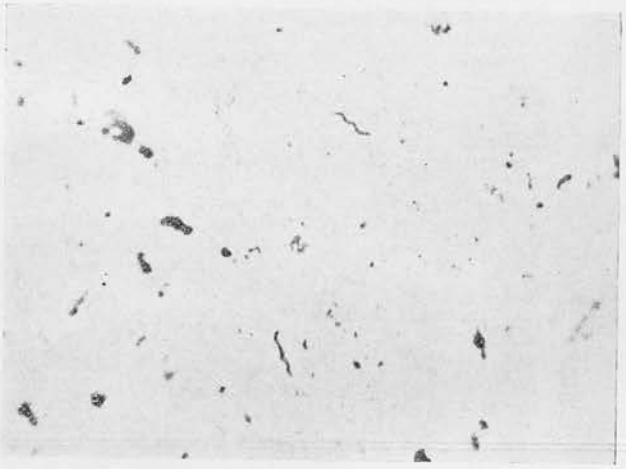

FIG. 1.

FIG. 2.

RESULTS

Br. abortus infection was detected in three animals from three herds. The strains recovered were fully virulent, distinct from strain 19; all three herds showed a history of brucellosis prior to vaccination. Apart from this, during

the present survey, the only organism which appeared to be of some significance was *V. foetus*. This organism was found in seven animals in three herds, *i.e.*, 30 per cent. of the foetuses examined, as shown in Table I.

three herds from which *V. foetus* was isolated in this investigation, one had no apparent infertility problem, the second had experienced no breeding difficulties until the present year and the infertility

TABLE I

DETAILS OF HERDS FROM WHICH *V. foetus* WAS OBTAINED

Herd No.	History of vaccination with S.19	Infertility problem	Name of cow	Period of gestation at which abortion occurred (months)	Presence of *Br. abortus* in any tissue examined (Biological tests)	Aggl. test for *Br. abortus*	Presence of *V. foetus* in foetal stomach	
							Smears	Cultures
1	Calfhood	None	Venelus	7	—	1/20	+	+ (first culture only)
2	Adult and calfhood, but terminated three years ago.	Present, but only this year, and other factors, *e.g.* bulls, involved.	Heather	6	—	1/80	+	+
			Leone	6	—	1/10	+	+
3	Calfhood and after every calving.	Present. Abortions increased from 2 per cent. to 8 per cent. from 1948 to 1950, and much infertility.	Naila	7	—	1/320	+	+ (first culture only)
			Margaret	7	—	1/160	+	+
			Success	5	—	1/40	+	Nil
		Conception rate, 3 : 1	Freedom	8	—	1/320	+	+

In the present survey the abortions due to *V. foetus* occurred between the fifth and eighth month of pregnancy, a fact of some significance in view of the common belief that this organism usually causes abortion in the first half of the gestation period. In smears from the tissues of the foetuses and also the cotyledons there was considerable variation in the number of organisms observed, and, as is well known, cultures were difficult to establish.

In one case no culture was obtained, despite the presence of *V. foetus* in smears from the foetal stomach, and in two cases the organism failed to grow on subculture after primary isolation. Two of the remaining three strains have been maintained on blood agar slopes for about five months, but these strains continue to show marked variation in growth on successive subcultures.

DISCUSSION

The object of the present work was to investigate possible causes of abortion in herds inoculated against brucellosis in Berkshire, and *V. foetus* was believed to be responsible for several cases. Since the work of Smith (1918) this infection in cattle has attracted considerable attention in many parts of the world, particularly in the U.S.A. (Plastridge & Williams 1943, Roberts, Gilman & Larsen 1950, and Moore 1950) and in Holland (Stegenga 1950); the general clinical and pathological features of this condition have been adequately dealt with by these and previous authors. In these two countries *V. foetus* has been incriminated as a major cause of abortion and also of infertility. Of the

in this herd is at present being investigated, but the information so far available suggests that *V. foetus* is probably not responsible in this instance. In the third herd a significant rise, from 2 per cent. to 8 per cent., occurred in the abortion rate over a period of two years from 1948 to 1950, despite the fact that vaccination against brucellosis commenced in 1946. Infertility was encountered and was associated with retained placentae, post-parturient discharges, stillbirths, weak calves which later died, and a low conception rate (three services per conception). *C. pyogenes* was previously isolated from this herd and had been accepted as a possible causal agent for these conditions.

V. foetus infection has only rarely been described in cattle in this country, *e.g.*, M'Fadyean and Stockman (1913) and McEwen (1940). This limited amount of information may, in all probability, be due to the previous high incidence of *Br. abortus* infection which masked most other causes of abortion. In addition, several difficulties are associated with the diagnosis of vibrionic abortion. So far the technique of the agglutination test has not been sufficiently developed to enable it to be used with real accuracy and a satisfactory stable antigen is yet to be prepared. Cultures are difficult to grow, frequently being lost during subcultivation, and although the technique of drying the primary isolations may to some extent eliminate this difficulty, no evidence is yet available. Accordingly, because of these difficulties, the 30 per cent. infection rate described in this paper, even although it appears somewhat

higher than the figures available from America, may, in fact, be considerably lower than the true incidence of the organism. Lovell (1950) has already drawn attention to similar condition where the distribution of an organism may be more widespread than hitherto believed. We would emphasise the fact that a laboratory diagnosis is essential in determining the presence of vibrionic abortion as the clinical picture is very similar to brucellosis and infertility cannot be attributed to *V. foetus* without adequate microscopic and cultural confirmation. In the present preliminary work no clear-cut association of the infection *per se* with infertility could be established, but the investigation will continue with this possible association in view. Moreover, quite apart from the abortions produced by *V. foetus* causing economic loss, and interfering with the interpretation of the results of vaccination with S. 19, this organism has recently been shown to be pathogenic for man (Vinzent, Delarue & Herbert 1950, Vinzent, Dumas & Picard 1947), and therefore it is essential that an effort should be made to determine the true extent and distribution of the organism in this country.

Summary

During an investigation into the possible causes of abortion in cattle previously vaccinated with S. 19 *Br. abortus*, it was found that *V. foetus* was present in seven aborted foetuses (30 per cent.) obtained from 20 he Berkshire.

Acknowledgment.—We wish to express our thanks D. L. Stewart, of the Reading Cattle Breeding Centr his interest in this work, and to Mr. F. Summerfield, technician of the Agricultural Research Council, Con for the photomicrograph.

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THE VACCINATION OF PREGNANT CATTLE WITH STRAIN 19 *BR. ABORTUS* VACCINE DURING AN OUTBREAK OF BRUCELLOSIS IN A DAIRY HERD

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During 1944 one of the three dairy herds (Cheseridge Farm) at this Field Station became infected with brucellosis. The circumstances under which this occurred were well defined. There was a severe outbreak of the disease at a neighbouring farm and animals from this herd gained access to Cheseridge by a public right of way. This occurred during the night and the presence of these cattle was not detected until early next morning when they were removed immediately. Apart from 15 reactors in first calf heifers on this farm during 1941-42, the Compton herds had been maintained free from brucellosis for many years, accompanied by a frequent blood-testing routine. In the present instance, after the contact with the infected cattle, it was only a matter of a few weeks before the first reactor was detected in this negative herd. In an ordinary commercial herd immediate vaccination would have been recommended, but we were anxious to maintain the animals completely free from infection so that susceptible cattle could be supplied from this farm for brucellosis experiments: therefore, a policy of blood testing, isolation and disposal of reactors was commenced. Immediately an animal failed to pass the agglutination test at 1 : 40 it was removed from the herd, but after numerous cows had been disposed of in this way, it became obvious that heavy loss would occur if this policy was continued and it was decided to vaccinate with S. 19.

Fig. 1 shows the result of the attempt made to control the disease by blood testing and elimination of reactors, from which it will be seen that in the course of approximately five months 48 reactors were detected.

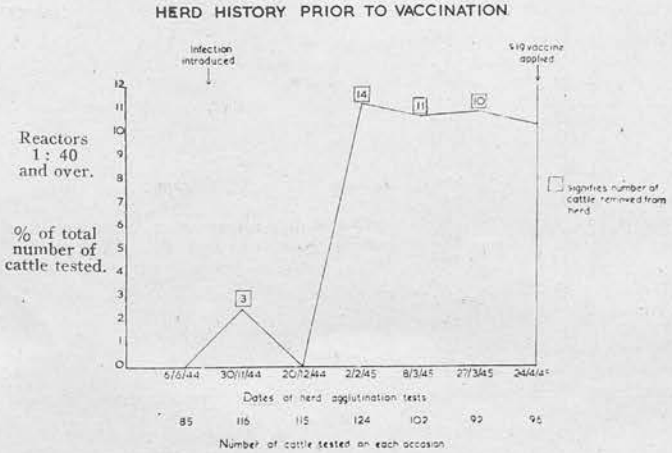

FIG. 1.

This experience showed that in the face of a rapidly spreading infection, blood testing, even at close intervals, with elimination of reactors, failed to arrest the progress of the disease. Consequently it was decided to vaccinate all the cows in the herd, irrespective of the stage of pregnancy, in an effort to create a satisfactory herd immunity.

Thus an opportunity was afforded to acquire information in the field regarding the vaccination of pregnant cows during an "abortion storm." The subsequent histories of the animals vaccinated during pregnancy and the cultural and pathogenic properties of strains of *Br. abortus* recovered at parturition, will be described in this paper.

MATERIALS AND METHODS

Animals Employed.—Forty-two Ayrshire and Friesian cows and heifers whose period of gestation at vaccination averaged 5·7 months. These animals were either negative to the agglutination test or reacting at 1 : 10 at the time of vaccination.

METHODS OF OBTAINING SAMPLES, CULTURAL AND AGGLUTINATION TECHNIQUE

These have been described in detail in previous papers, *e.g.*, McDiarmid (1949). At parturition cultures were prepared from the colostrum, the cotyledons, or, where these were unobtainable, from swabs taken from the cervix. The stomach contents of all the dead calves were also examined.

Ten *Br. abortus* colonies were picked from one of the original plates in order to provide a "mixed" culture which was preserved by drying and examined later.

Biological Tests.—If no growth of *Br. abortus* was observed on the plates within a period of two days the material was inoculated intramuscularly into two guinea-pigs of about 250 g. weight, the dose consisting of 2 ml. for each animal, 1 ml. being injected into each hind leg. After a period of three weeks the guinea-pigs were killed, blood was collected for the agglutination test and the spleens removed for examination for *Br. abortus*. The whole spleen was emulsified in twice its own volume of Ringer solution and 0·1 ml. of this suspension was spread upon the surface of a bacto-tryptose blood agar plate.

THE CLASSIFICATION OF STRAINS RECOVERED BY THESE METHODS

The strains were tested for:—

(1) CO_2 *Sensitivity.*

(2) *Sensitivity to Thionin Blue.*—McLeod's method (1944) was used.

(3) *Virulence for the Guinea-pig.*—The strains were examined for virulence by a method similar to that described by de Ropp (1945). Groups of guinea-pigs were injected intramuscularly with approximately 1,000,000 viable organisms suspended in Ringer solution and the subsequent course of infection was determined by killing animals at intervals of three, seven, and 15 weeks. The main index of infection was the presence of viable organisms in the spleen, but the percentage spleen weight to bodyweight and agglutination titre of the serum were also noted.

In all these tests suitable control cultures consisting of *Br. abortus* strain 19 and strain 544 were employed.

Procedure after Vaccination

The animals were bled and vaccinated subcutaneously with 5 ml. strain 19 vaccine on April 24th, 1944. Thereafter the following samples were obtained:—

Before Parturition.—Blood samples for agglutination test three weeks after vaccination and thereafter at three monthly intervals.

After Parturition.-

1. Blood.
2. Colostrum.
3. Cotyledon or swab from cervix.
4. Foetal stomach contents if available.

Results

A detailed history of each individual animal is given in Table I.

The Period of Gestation at which the Cattle were Vaccinated.—It will be seen from Table I that the period of pregnancy at which vaccine was applied varied considerably, the range extending from 12 to 236 days.

Agglutination Titres

(*a*) *At Vaccination.*—Twenty-nine animals, as shown in Table I, were completely negative to the agglutination test at vaccination and 13 showing a titre at 1 : 10 were recorded. Since all the cattle on the premises were exposed to the risk of *Br. abortus* infection, the possibility that some of these animals were in the early stages of infection could not be excluded and subsequent events showed that some animals were, in fact, infected prior to vaccination or at a later date.

(*b*) *Three Weeks after Vaccination.*—The total range of agglutination titres for all the vaccinated cattle was 1 : 20 to 1 : 5,120, the titres of those which were negative at vaccination ranged from 1 : 40 to 1 : 5,120, and the

Table I

Individual Histories of Pregnant Animals Vaccinated with Strain 19 *Br. abortus*

Serial No. of animal	Gestation period (at vaccn.) in days	Agglutination titres			History of pregnancy			Examination at parturition	
		At vaccn.	Three weeks later	Three months later	Gestation period (days)	Parturition	Fate of calf	Blood aggl. titre	Cultural and biological examination
23	178	10	20	10	276	N	L	10	
24	136	10	640	160	286	N	L	320	
36	164	—	640	80	282	N	L	40	*Cotyledons*
51	105	—	2,560	80	293	N	L	40	
54	184	10	1,280	10,240	265	P	L	5,120	Culturally, all were negative except
62	105	—	160	80	277	N	L	40	Nos. 65, 74 and 85. In addition,
65	196	10	640	10,240	244	A	D	5,120	Nos. 97 and 390 were positive bio-
71	209	—	320	40	284	N	L	80	logically.
74	198	10	1,280	2,560	221	A	D	1,280	
75	65	—	160	160	265	P	L	320	
82	180	—	640	40	277	N	L	40	
83	204	—	40	1,280	283	N	L	1,280	
85	159	10	40	160	172	A	D	160	
97	181	10	320	160	278	N (I)	L	80	
104	82	—	2,560	80	283	N	L	80	
106	197	—	640	40	285	N	L	40	
111	69	10	640	40	280	N	L	—	*Foetal stomach contents*
112	175	10	20	20	285	N	L	—	
117	127	—	640	40	289	N	L	40	Six samples were examined; all were
134	57	—	1,280	160	279	N	L	160	negative culturally except numbers 65
140	71	—	2,560	640	290	N	L	640	and 85. Cultures from No. 74 were
143	193	—	640	80	296	N	L	80	contaminated, but this sample was
147	189	—	2,560	320	278	N	L	320	positive biologically
328	153	—	320	2,560	254	A	L	1,280	
330	178	—	5,120	320	260	N	L	320	
336	186	10	640	80	282	N	L	40	
343	236	—	320	160	291	N	L	160	
350	12	—	5,120	160	289	N	L	320	
354	214	10	5,120	320	287	N	L	640	
365	205	—	5,120	1,280	287	N	L	1,280	
366	202	—	1,280	80	285	N	L	80	
367	168	—	5,120	320	280	N	L	320	
376	99	10	2,560	1,280	226	A	D	1,280	*Colostrum*
377	201	—	5,120	640	277	N	L	640	
378	207	—	1,280	320	283	dystokia	D	80	Culturally, all samples were negative
383	201	—	2,560	320	282	N	L	320	except Nos. 65 and 85. Biologically,
390	168	—	2,560	80	238	A	D	2,560	Nos. 74 and 97 were positive.
392	209	—	2,560	320	278	N	L	320	
394	209	10	5,120	5,120	286	N	L	2,560	
396	199	—	1,280	160	280	N	L	160	
381	166	—	2,560	160	275	N	L	160	
380	158	—	5,120	1,280	275	N	L	1,280	

— signifies Negative; (I) „ Infected; N signifies Normal; L „ Lived; P signifies Premature; D „ Dead; A signifies Abortion; 10 „ titre of 1 : 10

titres of those which had a primary titre of 1 : 10 ranged from 1 : 20 to 1 : 5,120.

(*c*) *Three Months after Vaccination.*—The range of agglutination titres for all the vaccinated animals was 1 : 10 to 1 : 10,240. For those which were originally non-reactors the range was 1 : 40 to 1 : 2,560, and the titres of those which reacted primarily at 1 : 10 ranged from 1 : 10 to 1 : 10,240.

(*d*) *At Parturition.*—The range for all the animals was 0 to 1: 5,120, the range of the original non-reactors was 1 : 40 to 1: 2,560 and the original 1 : 10 reactors showed a range of 0 to 1: 5,120.

History of Pregnancy

(*a*) *Gestation Period.*—This varied from 172 to 296 days (mean 273). The original non-reactor group showed a range of 238 to 296 days (mean 279) and the range of the original 1 : 10 reactors was 172 to 287 days (mean 261).

(*b*) *Living Calves.*—The total number of live calves produced from the 42 animals was 36 (85·7 per cent.). Of these calves 27 were born from the 29 original non-reactors and nine from the 13 cattle originally reacting at 1 : 10.

(*c*) *Infection Rate.*—Five of the 42 animals were found to be infected with *Br. abortus* at calving, the organisms being recovered from one or more of the tissues examined. Of these five cases, four belonged to the 1 : 10 group and one to the non-reactor group.

The Examination of Strains of *Br. abortus* Recovered from the Vaccinated Animals

Cultural Characters.—These are shown in Table II, and it will be observed that four of the five strains were CO_2 sensitive and capable of growth on thionin blue medium. The fifth strain (No. 390) was aerobic, highly sensitive to the presence of thionin blue and was presumably S. 19.

Virulence Tests in Guinea-pigs

The results of virulence tests are shown in Table III. In the S. 19 (control group) infection was shown to be present three weeks after the guinea-pigs had been inoculated, a trace of infection was shown to be present at seven weeks and no infection was detected at 15 weeks. In the 544 group (controls) persisting infection was found at three, seven, and 15 weeks. The spleen weight-bodyweight ratios and agglutination titres were correspondingly greater in the 544 group.

Table II

Cultural Examination

Strain No.	CO_2 sensitivity	Thionin blue sensitivity
65	+	—
74	+	—
85	+	—
97	+	—
390	—	+
Controls		
S. 19	—	+
544	+	—

With one exception, No. 390, strains isolated from the pregnant cows conformed to the 544 pattern producing persisting infection, high titres and large spleens in the guinea-pigs. Strain 390 was similar to S. 19 in that, although the organism was recovered at three weeks from the spleens of the experimental animals, no evidence of infection was found at seven or 15 weeks. Moreover, the spleens of these guinea-pigs were not altered in size and the agglutination titres remained consistently low.

Discussion

An examination of the literature does not reveal many published accounts of clear-cut experiments to determine the effect of inoculating pregnant cows with S. 19 *Br. abortus* and the evidence available is somewhat conflicting. Haring and Traum (1937) reported that they vaccinated eight pregnant cows with S. 19. Subsequently three of these animals were exposed to a virulent experimental infection—a strain of *Br. abortus* resembling S. 19 was recovered from one animal in each group at parturition. Birch, Gilman and Stone (1943) fed S. 19 at frequent intervals to eight cows; one animal produced a premature calf and the placenta was proved infective by biological examination. Deem and Cross (1945) from experience in the field give two examples of abortion following vaccination with S. 19. Moore and Mitchell (1948) report the

Table III

Virulence Tests in Guinea-pigs

Strain number	Mean agglutination titre			Mean spleen weight/Bodyweight ratio *			Spleen cultures		
	3 weeks	7 weeks	15 weeks	3 weeks	7 weeks	15 weeks	3 weeks	7 weeks	15 weeks
65	384	7,680	10,240	0·17	0·41	0·39	+ 5/5	+ 5/5	+ 5/5
74	256	3,408	10,240	0·21	0·27	0·44	+ 5/5	+ 5/5	+ 5/5
85	344	7,680	7,168	0·14	0·79	0·34	+ 5/5	+ 5/5	+ 5/5
390	186	14	0	0·14	0·14	0·13	+ 5/5	—	—
Controls									
544	448	10,240	7,168	0·20	0·44	0·32	+ 5/5	+ 5/5	+ 5/5
S 19	64	16	0	0·13	0·14	0 14	+ 5/5	+ 2/5	—

* signifies Mean for five guinea-pigs.
\+ „ *Br. abortus* isolated.
— „ No *Br. abortus* isolated.
5/5 „ Five out of five guinea-pigs infected.

The field strain from cow No. 97 failed to grow from the dried state, and therefore no biological tests were made on this culture.

vaccination of 68 pregnant animals, all of which calved normally. Subsequently, the same authors (1950) vaccinated an additional 30 pregnant cows—*Br. abortus* was not recovered at parturition. In the present work cultural and biological examinations were carried out with material taken at parturition from cattle vaccinated during pregnancy with S. 19. These cattle were exposed to the risk of natural infection with *Br. abortus* before and after vaccination, but in view of the work of Mingle, Manthei and Jasmin (1941) and Taylor and McDiarmid (1949), the stability of the character of reduced virulence of S. 19 was considered to be of sufficient value to distinguish this strain from any field strains which might be encountered. Throughout the course of this work only one animal of 42 examined was found to be infected with S. 19 at parturition. In view of the fact that the range of duration of pregnancy when the vaccine was applied varied from 12 to 236 days with a mean gestation period of 160 days, it appears that the vaccination of pregnant cattle at any period of pregnancy is justified when dealing with a severe outbreak of contagious abortion in a dairy herd. Moreover, in an infected herd, the possibility always exists that non-immunised pregnant animals might become infected with the field strains with subsequent infective abortions, whereas if an abortion should occur in a pregnant animal as a result of vaccination with S. 19 the evidence available suggests that this strain will remain unaltered in character and the danger of spread of infection will be materially reduced.

Summary

An attempt was made to control the spread of *Br. abortus* infection in a dairy herd by blood testing and disposal of reactors. This met with no success and it became necessary to vaccinate all the cows, including pregnant animals, with S. 19 vaccine.

Five cases of *Br. abortus* infection were detected at parturition from 42 animals vaccinated at a mean gestation period of 5·7 months. Four of these five strains were apparently field strains, but the fifth strain was aerobic, sensitive to thionin blue, and similar to S. 19 in virulence for the guinea-pig. Our experience indicates that the risk of abortion due to vaccination of pregnant cattle with S. 19 *Br. abortus* vaccine appears to be slight. The findings of other authors are reviewed.

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THE OCCURRENCE OF AGGLUTININS FOR *BR. ABORTUS* IN THE BLOOD OF WILD DEER IN THE SOUTH OF ENGLAND

BY

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Evidence is accumulating to show that various species of free-living wild animals may be infected with brucellosis, and the majority of recorded cases have occurred in elk, bison and buffalo, principally in the National Parks of America (Katz, 1941). In this country, Bosworth (1937) isolated *Br. abortus* from a wild rat, but this appears to be the only record of this particular infection in a free-living wild animal in Britain. In a general study of the epidemiology of brucellosis, and the development of a control policy for the eventual elimination of contagious abortion, it is essential to know if natural reservoirs of infection exist in animals other than domestic cattle. An opportunity occurred to examine the blood sera of deer, and the results are recorded in this paper.

MATERIALS AND METHODS

Type of Deer Available.—A total of 80 deer of three different species were examined, *viz.*:—

Fallow (*Dama dama*).

Japanese (*Sika nippon*).

Roe (*Capreolus capreolus*).

These were obtained from seven different counties, namely, Berkshire, Wiltshire, Hampshire, Gloucestershire, Shropshire, Herefordshire, and Dorset.

Blood Samples.—With the kind co-operation of the Forestry Commission, Agricultural Executive Committees and local gamekeepers, it was arranged that during the winter months of two years, 1948-50, blood samples would be collected by personnel engaged in the shoots that are essential for the control of the deer population in densely wooded areas. Immediately a deer was shot, blood was collected from the jugular vein into McCartney bottles and the species, age (if known), sex, and place of killing recorded.

Agglutination Tests.—These were performed according to the standard technique for cattle, using the *Br. abortus* antigen of the Ministry of Agriculture.

RESULTS

The data relating to the origin of samples and the results obtained are shown in Table I. Of the 80 samples examined, eleven (13·8 per cent.) contained demonstrable agglutinins for *Br. abortus*. Of these eleven samples, five reacted at 1 : 40, four at 1 : 20, and two at 1 : 10. All were obtained from fallow and Japanese deer—none of the 17 samples from roe deer was found to be positive.

TABLE I

THE EXAMINATION OF BLOOD FROM WILD DEER FOR *Br. abortus* AGGLUTININS

Species	Deer No.	Source	Age	Sex	Agglutination titre			
					N.	1 : 10	1 : 20	1 : 40	1 : 80
	1	Hampshire	2 yrs.	M.					
	2	„	4 „	F.			+		
	3	„	6 „	F.					
	4	„	5 „	F.					
	5	„	1 yr.	M.					
	6	„	8 yrs.	F.					
	7	„	1 yr.	F.					
	8	Shropshire	?	F.		+			
	9	„	?	F.					
	10	„	?	F.					
	11	„	?	M.					
	12	„	?	M.					
	13	„	?	F.					
	14	„	?	M.					
	15	„	?	F.					
	16	„	?	M.					
	17	„	?	M.					
	18	„	?	M.					
	19	„	?	M.					
	20	„	?	M.					
	21	Hampshire	9 mths.	M.					
	22	„	5 yrs.	F.					
	23	Herefordshire	?	F.					
	24	„	?	F.					+
	25	„	1 yr.	M.					
	26	„	?	F.					
	27	„	?	F.			+		
	28	„	?	M.					
FALLOW (*Dama dama*)	29	„	?	M.					
	30	„	Fawn	F.			+		
	31	„	?	F.					
	32	„	1 yr.	M.			+		
	33	„	Young	F.					
	34	„	„	M.					
	35	„	?	F.					+
	36	Hampshire	6 yrs.	M.					
	37	„	7 „	M.					
	38	Berkshire	2 „	M.					
	39	Hampshire	4 „	M.					
	40	„	1 yr.	F.					
	41	„	4 yrs.	F.					
	42	„	1 yr.	M.					
	43	Berkshire	2 yrs.	M.					
	44	„	2 „	F.					
	45	Hampshire	10 „	M.					
	46	„	8 „	M.					
	47	„	4 „	F.					
	48	„	2 „	F.					
	49	Dorset	?	M.					
	50	„	?	F.					
	51	„	?	?					
	52	Gloucestershire	2 yrs.	F.					
	53	„	2 „	F.					
	54	„	2 „	F.					
	55	„	2 „	M.					
	56	Berkshire	3 „	F.					
	57	Gloucestershire	7 „	M.					
	58	„	1 yr.	F.					

Summary of results: 51 negative, 1 at 1 : 10, 4 at 1 : 20, 2 at 1 : 40

Species	Deer No.	Source	Age	Sex	Agglutination titre N.	1 : 10	1 : 20	1 : 40	1 : 80
	59	Hampshire	8 yrs.	M.					
	60	„	4 „	F.					
	61	„	3 „	F.					
	62	„	2 „	M.					
	63	„	11 „	M.					
	64	„	6 „	M.					
	65	„	2 „	M.					
Roe	66	„	2 „	M.					
(*C.*	67	„	5 „	F.					
capreolus)	68	„	2 „	F.					
	69	„	3 „	F.					
	70	„	4 „	M.					
	71	Dorset	?	M.					
	72	„	?	M.					
	73	„	?	F.					
	74	„	?	F.					
	75	„	?	?					
Summary of results : All negative.									
	76	Hampshire	7 yrs.	M.					+
Japanese	77	„	2 „	F.					+
(*Sika*	78	Dorset	5 „	M.					
nippon)	79	„	?	F.					+
	80	„	?	F.		+			
Summary of results : 1 negative, 1 at 1 : 10, 3 at 1 : 40.									

Discussion

If the significance of agglutination titres occurring in the blood of deer can be interpreted in the same way as in cattle, then this preliminary survey would indicate the occurrence of *Br. abortus* infection in five out of 80 deer. Four would be classified as doubtful reactors and 71 as negative. The absence of 1 : 10 reactors, apart from two cases, is somewhat contrary to the usual findings in cattle living in an abortion-free herd where the proportion of 1 : 10 reactors is seldom below 9 per cent. (McDiarmid, unpublished observations). Moreover, it is interesting to compare the present results with those of Taylor (1939), who examined the blood of farm horses in Scotland for *Br. abortus* agglutinins. Of the 957 sera which he examined, 19·6 per cent. reacted at 1 : 10, 2·1 per cent. at 1 : 20, and 1·2 per cent. a 1 : 40. In the present work the corresponding figures for 80 sera examined were: 2·5 per cent. at 1 : 10, 5·0 per cent. at 1 : 20, and 6·3 per cent. at 1 : 40. It is interesting to note that, so far, no reactors have been detected in roe deer. This species is a true British wild mammal, whereas most of the fallow and all the Japanese deer now breeding in the Forestry plantations probably originated from stock escaped from deer parks. Moreover, the retiring habits of roe tend to segregate them from domestic animals and consequently from sources of infection to a greater extent than the other two species.

It is unfortunate that circumstances would not permit the cultural and biological examination of tissues from the reactors, but it is hoped that, in future work, material will be available for this purpose.

Summary

Eighty blood samples from three species of wild deer in the South of England were examined for *Br. abortus* agglutinins; five reacted at 1 : 40, four at 1 : 20, and two at 1 : 10.

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A Comparison of the Intradermal and Subcutaneous Routes in producing Immunity to Brucellosis in Cattle.

by

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During the last ten years, several experiments to investigate immunity against brucellosis have been conducted at this Field Station; (Edwards, de Ropp and McLeod 1945; Edwards, McDiarmid, de Ropp and McLeod 1946; McDiarmid 1949, 1950). Using the standard technique adopted in these experiments McDiarmid (1950) showed that 0.2 ml. of S. 19 vaccine, given by the intradermal route, created an immunity equal to that produced by a large dose (5.0 ml.) injected subcutaneously. When this preliminary experiment was commenced sufficient cattle were not available for the inclusion of a group vaccinated with the same amount namely 0.2 ml. S. 19 by both routes, although it was fully realised that such a comparison with the same dosage should have been made. Later further experimental animals became available and this paper describes the results of comparing the immunity produced by this small dose injected by both routes with the more commonly employed dose of 5.0 ml. subcutaneously.

Materials and Methods.

Animals.

These consisted of forty Ayrshire heifers reared in a

non-vaccinated brucellosis-free herd and all gave negative results to the agglutination test on two occasions prior to vaccination. The heifers were about two months in calf at the time of vaccination and in this respect differed from animals, used in previous experiments, which had been vaccinated prior to service; four groups were formed, ten animals in each group, and these were housed in separate isolation units of the type described in previous papers, e.g. Edwards, et.al. (1946).

Vaccination.

Strain 19 Br. abortus vaccine (Ministry of Agriculture standard issue) was inoculated as follows:-

Group 1. (10 heifers) Subcutaneous route (Small Dose).

A dose of 0.2 ml. was injected subcutaneously into the thoracic region just posterior to the olecranon process.

Group 2. (10 heifers) Intradermal route.

A dose of 0.2 ml. was injected intradermally at the same inoculation site as in Group 1.

Group 3. (10 heifers) Subcutaneous route. (Large dose)

A dose of 5.0 ml. was inoculated subcutaneously in the same position as used for the previous two groups. This group was included in order to determine the protective power of the vaccine, i.e. used in a dose which already had proved effective in past experiments at this Field Station.

Group 4. (10 heifers) Controls. (Non-vaccinated).

These animals formed a control on the infectivity of

the test dose.

Exposure to Infection.

The heifers were approximately five months in calf, i.e. three months after vaccination, at the time of infection. The same procedure was adopted as in previous experiments i.e. 0.1 ml. of a suspension estimated to contain approximately 150 million viable Br. abortus, strain 544 was instilled into the conjunctival sac of each animal. The viability and virulence of this infecting suspension was checked by plate counts and by inoculation of guinea pigs. The results of the tests in guinea pigs are shown in Table 1, and the actual viable count showed the infective dose to contain 134 million organisms.

Agglutination Tests.

Blood samples from the heifers were examined frequently for the presence of agglutinins. The technique was similar to that used on previous occasions e.g. Edwards et al. (1946)

Examination of Materials Collected at Parturition.

This closely followed the technique described in our previous papers; cotyledons, foetal stomach contents, when available, colostrum and milk samples were examined culturally and biologically; in addition, the blood sero-agglutination titre of each animal was recorded at parturition. Milk samples from the individual animals were tested for the presence of Br. abortus each week for a period of ten weeks after calving.

RESULTS.

I. General Response to Vaccination.

Small Dose (0.2 ml.) subcutaneously.

In this group no marked systemic reactions were noted. The local oedematous area varied from 1" to 2" in diameter - small in comparison with the typical picture following the injection of 5.0 ml., one of the heifers showed no abnormality at the inoculation site.

Small dose (0.2 ml.) intradermally.

The reactions associated with this type of injection have already been described in detail, McDiarmid (1950) and the present results obtained in this group confirmed these earlier findings; A small local necrotic lesion which healed rapidly, was produced at the inoculation site.

Large dose (5.0 ml.)subcutaneously.

In the majority of cases, the local and systemic reactions following inoculation, were typical of those usually produced by this particular dose. These reactions which are much larger than those associated with the small dosage have been described previously (Edwards et al. 1946, McDiarmid 1949, 1950). Two heifers showed no apparent reactions.

II. Immunity produced.

The results obtained following the application of the infective dose are shown in detail in Table 2 and summarised in Table 3. Figure 1 depicts the mean agglutination titres in the different groups throughout the course of the experiment.

(a) Subcutaneous Group (Small Dose)

By this method of vaccination, protection against the test

dose was afforded to five heifers out of ten. It is interesting to note that one animal, eventually proved to be immune, (No. 95350), had failed to respond to vaccination in a satisfactory manner when judged by the agglutination titre. On no occasion subsequent to vaccination did the titre of this particular heifer rise above 1 in 40. The five animals infected in this group produced four dead calves and infection was detected in four of the cotyledon samples, two of the samples from foetal stomachs and all the colostrum samples. Nineteen per cent. of the post partum milk samples were infected.

(b) Intradermal Group.

A similar protection rate was obtained as in the previous group, four cotyledons, two foetal stomach samples and two colostrum samples were found infected from five infected heifers - only two dead calves in this group could be attributed to Br. abortus infection. The milk was comparatively free from infection, compared with the other three groups, six out of ninety samples containing Br. abortus (6.7 per cent.).

(c) Subcutaneous Group (Large Dose).

The protective rate was again of the same order as in the previous two groups, namely five out of ten animals; cotyledons from all five infected animals were shown to contain Br. abortus and in addition, the stomach contents

of three out of three dead calves were found infected. Only two colostrum samples were positive. The incidence of excretion of Br. abortus in the milk (23 per cent.), closely corresponded to the figure obtained in the group vaccinated with the smaller dose by the same route.

With one exception, all strains of Br. abortus isolated from the experimental heifers were CO_2 sensitive and produced persisting infection in guinea pigs similar to that produced by strain 544. In heifer No. 25992 the only strain isolated at parturition was aerobic, highly sensitive to thionin blue and of reduced virulence for guinea pigs. These findings support the belief that S.19 was responsible for the abortion in this instance.

(d) Controls. Non vaccinated Group.

The infective dose used in this experiment appeared to be of a satisfactory nature since all ten animals showed evidence of infection at the time of parturition. Br. abortus was isolated from the cotyledons in every case, from the foetal stomachs in seven of the calves and from the colostrum of every heifer. All ten calves were born dead. Thirty-three per cent. of post-partum milk samples were infected.

DISCUSSION

Previous findings, McDiarmid (1950) confirmed the theory of Campbell and Rodwell (1945) that a small dose of S.19 vaccine given intradermally would produce a satisfactory degree of immunity to brucellosis. It was not possible

to assemble sufficient animals for this preliminary experiment to prove whether the route per se was wholly responsible for the successful response to this dose of antigen, or that the same number of organisms given subcutaneously would have produced a similar result. In the present experiment the immunity produced by 0.2 ml. S.19 injected intradermally was shown to be equal to that obtained by the same quantity of vaccine given subcutaneously but in both groups and in the group vaccinated with 5.0 ml. the level of immunity was poor in comparison with previous results only five of the ten vaccinated animals being protected compared with the usual eight.

Various suggestions can be presented to account for this result. At the commencement of the present experiment pregnant heifers were used, owing to a scarcity of non-pregnant animals and a desire to obtain an answer to this question at an early date because of further work contemplated to explore the duration of immunity following vaccination. It is feasible that the pregnant heifer does not respond so well to the introduction of Br. abortus antigen as does the non-pregnant animal and that consequently the antibody level is correspondingly lower. Another factor which must be considered is the shorter interval between the time of vaccination and the application of the challenge dose; it could be deduced that this had to some extent influenced the final outcome of this experiment. The efficiency of this batch

of vaccine was carefully checked by viable plate counts and by the inoculation of guinea pigs at Weybridge prior to issue and was apparently quite satisfactory with regard to the number of viable organisms present and the subsequent protection afforded to the guinea pigs. Its protective power in cattle was, of course, not known prior to this experiment. If it was purely a question of viable organisms present in the vaccine, a better protection rate could perhaps have been expected in the group inoculated with 5.0 ml. compared with the smaller dose groups since this dose contains approximately twenty five times the number of organisms. The infective dose was carefully checked by viable count and by inoculation of experimental animals and was in fact lower than the estimated amount. The possibility that immunity had been overwhelmed by an excessive test dose can therefore be excluded.

It is extremely difficult to decide which factor or factors were responsible for the results obtained. It can only be stressed that the two main deviations in this experiment from our previous technique were the use of animals already pregnant at vaccination and a diminished interval between the immunisation of the heifers and the application of the test dose; it must however, be borne in mind that the amount of evidence available concerning the application of this particular test dose to vaccinated cattle is still somewhat limited. The recovery of only one strain of Br. abortus which possessed the character of reduced virulence

usually associated with S.19 again confirms the author's previous findings, McDiarmid (1951) that vaccination of pregnant cattle can be carried out without undue risk of the animals aborting due to vaccination. This is of considerable importance when dealing with an abortion storm in a non-vaccinated herd.

SUMMARY

A small dose (0.2 ml.) of S.19 Br. abortus vaccine administered subcutaneously was shown to confer an immunity to heifers in no way inferior to that produced by the same dose intradermally or by a larger dose (5.0ml.) by the subcutaneous route. The protective power of the vaccine did not appear to be as effective as in previous experiments - various factors are discussed which may have been responsible for this phenomenon.

ACKNOWLEDGEMENTS

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Fig.1.

MEAN AGGLUTINATION TITRES.

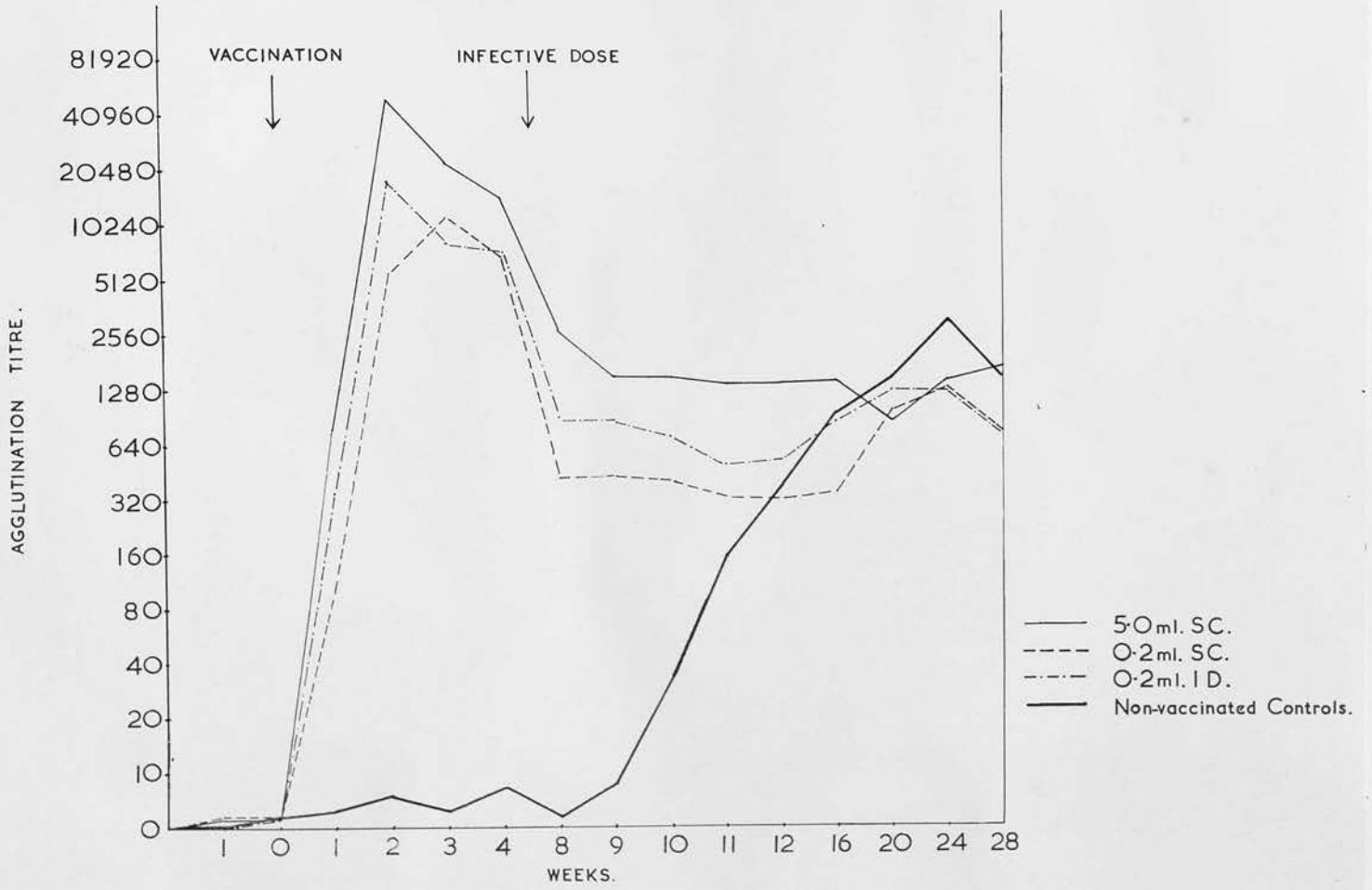

TABLE 1.

Virulence Test of Infective Dose.

10 Guinea pigs inoculated intramuscularly with 3×10^6 strain 544.

16 days			9 weeks		
Agglutination titre	% Spleen wt./ Body wt.	Approximate number of *Br. abortus* in spleen	Agglutination titre	% Spleen wt./ Body wt.	Approximate number of *Br. abortus* in spleen
2560	0.16	265,000	81920	2.8	13,000
1280	0.19	175,000	20480	0.45	2,000
2560	0.19	198,000	5120	0.20	3,000
1280	0.14	180,000	1280	0.16	4,000
1280	0.19	142,000	Died	-	-

TABLE 2.

COMPARISON OF IMMUNITY PRODUCED BY VARIOUS DOSES OF S.19 VACCINE ADMINISTERED BY DIFFERENT ROUTES.

Group	Number of Heifer	Service to vaccination (days)	Agglutination titres of serum: Weeks prior to vaccination		Weeks after vaccination				Infective dose applied	Weeks after infection							History of Pregnancy: Service to Infection (days)	Infection to Parturition (Days)	Duration of Pregnancy (Days)	Parturition	Fate of calf	Examination at Parturition: Blood Serum Titre	Cotyledons		Foetal Stomach		Colostrum	
			1	0	1	2	3	4	8	4	8	12	16	20	24	28							Cult.	Biol.	Cult.	Biol.	Cult.	Biol.
1	49502	99	N	N	4	8	8	7	4	8	8	10	11	10			159	82	241	TA	D	10	+	+ 5/5	C	N 0/5	N	+ 5/5
0.2ml.	49464	121	N	N	3	8	6	6	3	4	5	8	7	7			181	79	260	TA	D	8	+	+ 5/5	+	+ 5/5	N	+ 5/5
S.C.	54810	106	N	N	5	9	9	9	3	5	5	5	5	4			166	87	253	P	L	5	+	+ 5/5	..	..	N	+ 4/5
	95350	106	N	N	2	3	3	3	1	1	1	N	1	1	1		166	116	282	Nl	L	1	N	N 0/5	..	..	N	N 0/5
	1418	95	N	N	5	8	8	7	4	4	4	4	4	4	5	5	155	121	276	Nl	L	5	N	N 0/5	..	..	N	N 0/5
	54803	81	1	1	3	12	12	12	9	7	8	9	9	8			141	87	228	TA	D	9	+	GPD	C	N 0/3	+	+ 2/5
	26008	80	1	1	4	8	8	7	5	7	6	6	6	5	9		140	108	248	TA	D	6	+	+ 5/5	+	+ 4/4	N	+ 4/4
	70351	79	N	N	5	9	10	8	3	3	3	2	2	2	2	2	139	141	280	Nl	L	2	C	N 0/5	..	..	C	N 0/4
	54828	75	1	1	5	12	14	13	8	6	6	6	6	7	7	7	135	138	273	Nl	L	7	N	N 0/5	..	..	N	N 0/5
	545	91	1	1	4	6	3	5	2	1	N	1	1	1	1		151	119	270	Nl	L	1	N	N 0/5	..	..	N	N 0/5
2	54796	101	N	1	6	13	11	9	7	7	9	9	10	9	7		161	54	215	TA	D	8	N	..	+	+ 5/5	+	+ 5/5
0.2ml.	482	101	N	N	6	10	9	10	7	7	7	8	6	6	7	7	161	119	280	NL	L	6	N	N 0/5	..	..	N	N 0/5
I.D.	520	95	N	N	6	9	9	8	4	3	3	3	3	3	4	3	155	129	284	Nl	L	3	N	N 0/4	..	..	N	N 0/5
	25974	101	N	N	7	10	10	9	6	6	6	7	6	6			161	70	231	TA	D	8	+	+ 5/5	N	+ 5/5	N	N 0/5
	541	106	N	N	7	11	11	9	6	6	7	8	6	6			162	86	248	P	L	8	+	+ 5/5	..	..	N	N 0/5
	25992	102	N	N	5	10	10	8	6	6	8	10	10	8	8		166	106	272	P	L	10	+	+ 2/5	..	..	N	N 0/5
	70345	102	N	N	6	12	12	11	10	8	7	7	7	7	7		162	119	281	Nl	L	7	N	N 0/5	..	..	N	N 0/5
	49493	73	N	1	5	14	12	13	8	8	8	8	7	7	8	8	133	147	280	Nl	L	7	..	N 0/3	..	..	C	N 0/3
	26013	74	N	1	6	10	10	10	6	6	6	6	7	7	7	7	134	142	276	Nl	L	7	C	+ 4/4	..	..	C	+ 3/5
	81656	83	N	N	6	8	6	6	3	2	2	2	2	Dead			143	136	279	Dystokia	D	3	C	N 0/5	..	GPD	..	N 0/5
3	54788	99	N	1	7	13	11	13	10	7	7	8	8	8	6		159	106	265	TA	D	8	+	+ 5/5	+	+ 4/4	N	N 0/5
0.0ml.	26016	94	N	N	7	11	12	11	9	6	6	7	8	10	11		154	109	263	P	L	8	+	+ 5/5	..	..	+	+ 5/5
S.C.	70346	101	N	N	6	13	11	11	9	7	8	7	7	7	8	8	161	122	283	Nl	L	8	N	N 0/5	..	..	N	N 0/5
	534	101	1	N	6	10	10	9	8	6	6	6	6	6			161	116	277	Nl	L	6	N	N 0/5	..	..	N	N 0/5
	70356	105	N	N	8	12	10	9	7	6	7	8	8	8			165	70	235	TA	D	8	..	+ 4/4	N	+ 5/5	N	N 0/5
	81652	89	1	N	8	11	11	12	7	6	6	6	5	5	7	6	149	126	275	Nl	L	6	N	N 0/5	..	..	N	N 0/5
	49509	89	N	N	7	13	12	11	8	8	8	8	10	10	12		149	96	245	P	L	9	+	+ 4/4	..	..	N	+ 5/5
	529	92	N	N	8	12	13	10	8	6	6	6	6	6	7	7	152	123	275	Nl	L	7	N	N 0/5	..	..	N	N 0/5
	533	92	N	1	7	16	14	13	11	8	8	7	7	7	8	8	152	133	285	Nl	L	7	N	N 0/4	..	..	N	N 0/5
	539	71	1	1	6	12	12	11	8	11	11	9	10	9	8		131	105	236	TA	D	10	+	+ 1/4	+	+ 3/5	N	N 0/5
4	49468	96	N	N	N	N	N	1	N	3	5	7	6	6			156	65	221	TA	D	8	N	+ 3/3	N	N 0/3	C	+ 2/3
N.V.	54815	95	N	N	N	N	N	N	N	3	2	5	7	7	8		155	111	266	TA	D	7	C	+ 3/3	C	N 0/3	N	+ 2/2
Controls	536	99	N	1	N	1	N	N	N	5	5	8	8	7			159	66	225	TA	D	8	+	..	+	..	N	+ 3/3
	49480	100	1	1	1	1	1	1	1	9	10	10	8	8			160	67	227	TA	D	14	+	..	+	..	C	+ 3/3
	542	95	N	N	N	1	N	1	N	6	9	10	12	11			155	70	225	TA	D	14	C	+ 3/3	+	..	+	..
	70319	101	N	N	N	N	N	N	N	5	5	6	7	7			161	58	219	TA	D	5	+	..	+	..	N	+ 1/3
	78429	88	N	N	N	N	N	1	N	4	7	9	9	8			148	76	224	TA	D	11	+	..	+	..	N	+ 3/3
	70348	75	N	N	1	1	1	1	N	5	4	4	3	2			135	55	190	TA	D	4	+	..	+	..	N	+ 2/3
	54812	71	N	1	N	1	N	1	1	3	5	6	8	7			131	84	215	TA	D	6	C	+ 3/3	N	N 0/3	N	+ 1/3
	25983	70	N	1	1	1	1	1	N	5	5	8	8	8			130	78	208	TA	D	7	+	..	+	..	N	+ 3/3

.. signifies Not done
NV " Non vaccinated
I.D. " Intradermally
TA " Typical Abortion
N " Negative
C " Contaminated

Nl signifies Normal
GPD " Guinea pigs died
L " Living
P " Premature

N 0/5 signifies Five pigs negative
S.C. " Subcutaneously
D " Dead
+ " Positive

The agglutination titres are expressed by tube numbers
e.g. 1 = 1/10, 2 = 1/20, 3 = 1/40, etc.

TABLE 3.

Summary of Results.

Group	Method of Vaccination	Mean duration of pregnancy	Number of Heifers infected	Number of living calves	Excretion of *Br. abortus* in the milk	
					Number of samples examined	Number of samples positive
1 10 heifers	0.2ml. S.19 vaccine sub-cutaneously	261	5	6	100	19
2 10 heifers	0.2ml. S.19 vaccine intradermally	265	5	7	90	6
3 10 heifers	5.0ml. S.19 vaccine sub-cutaneously	264	5	7	100	23
4 10 heifers	Non-vaccin-ated controls	222	10	0	100	33

A. McDiarmid.

Assessing the Immunising Value of Br. abortus Vaccines in Cattle.

Assessing the Immunising Value of Br. abortus Vaccines in Cattle.

A. McDiarmid.

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In Britain considerable progress has been made during the last ten years towards the control and eventual elimination of brucellosis in cattle. The incidence of the clinical disease is now below 4 % in many areas and this figure includes abortions due to a variety of causes quite apart from *Br. abortus* infection. The disease is, in fact, no longer of any economic importance to the farmer. This state of affairs has been brought about chiefly by widespread vaccination with S. 19 — the test and slaughter policy as practised in some other countries being quite inapplicable to Britain because of the dense cattle population and the high initial level of infection. The practice of vaccination has been built up gradually over a considerable period. As long ago as 1932, experiments in guinea-pigs were in progress at Moredun Institute to try to determine if vaccination with attenuated strains of *Br. abortus* would be a practical proposition. The technique of this preliminary method of assessing the value of vaccines was adopted by other research institutes in Britain and the culminating result was the accumulation of a vast amount of information pertaining to many aspects of immunity. Soon it became clear that the results obtained with strain 45/20 (Mc Ewen) and the American S. 19 fully justified the extension of the work with both these vaccines to cattle and it was felt that the only way of assessing the true value of either vaccine would

be under conditions of strict isolation and controlled experimentation; this was one of the main reasons why, in 1937, the Agricultural Research Council purchased an estate at Compton in Berkshire as an experimental field station to provide suitable cattle bred in a disease free environment and isolation accommodation, not available elsewhere, to house them. The object of the present paper is to describe briefly the technique used at Compton for testing *Br. abortus* antigens in cattle and to draw attention to the type of work at present in progress.

Three main conditions are necessary if reliable results are to be obtained:

(1) Adequate isolation accommodation.

(2) Suitable susceptible experimental cattle.

(3) The ability to produce at will, a disease indistinguishable from the natural infection.

All these conditions can be fulfilled at Compton.

1. *Accommodation.*

The original type of building used for this class of work is shown in Fig. 1 and in the initial stages six of these were built, each housing ten animals. Recently, because of the foresight and initiative of Dr. W. S. Gordon, Director of the Field Station at Compton, a new isolation compound has been constructed (Fig. 2). Forty-four separate buildings each containing twelve animals, are now available. Thus over 500 cattle can be housed in this compound. The new type of isolation unit is shown in Fig. 3. Each individual unit consists of a main shed, an exercising yard, calving box and food store. In addition a changing room is provided where personnel can be suitably prepared before entering or leaving the unit. The cattle are machine milked and separate sterilising and washing up rooms are provided for each unit. The whole building is bird proofed to minimise the risk of spread of infection from extraneous sources to the experimental cattle and from any infected cows within the units to susceptible normal animals in the immediate environment. An abbatoir, laboratories, bull pens, AI Centre, and offices are also provided.

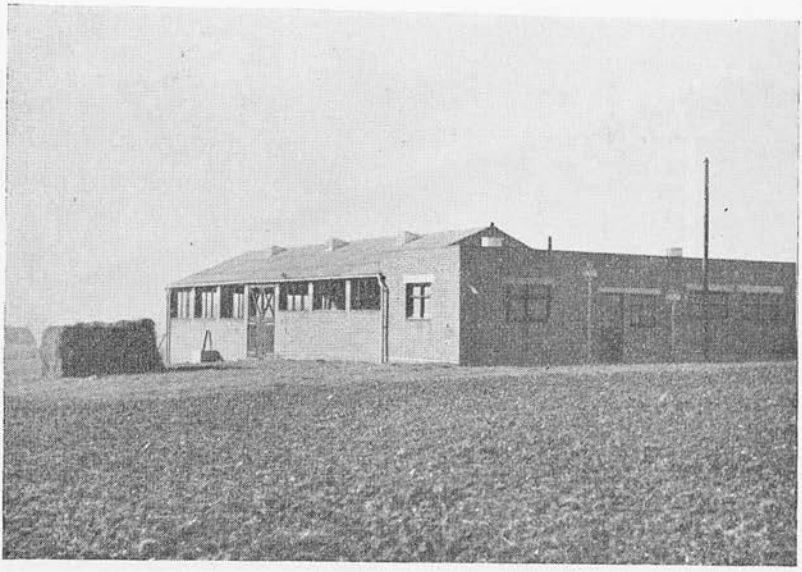

Fig. 1. Original type of isolation unit.

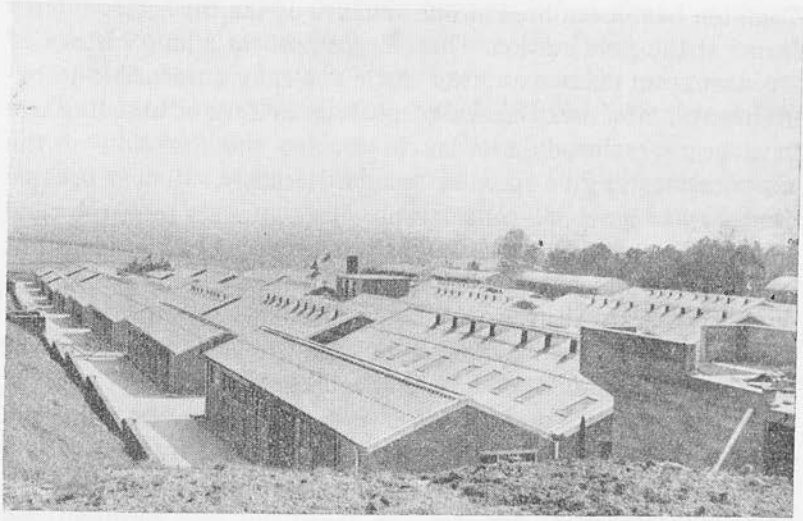

Fig. 2. New isolation compound.

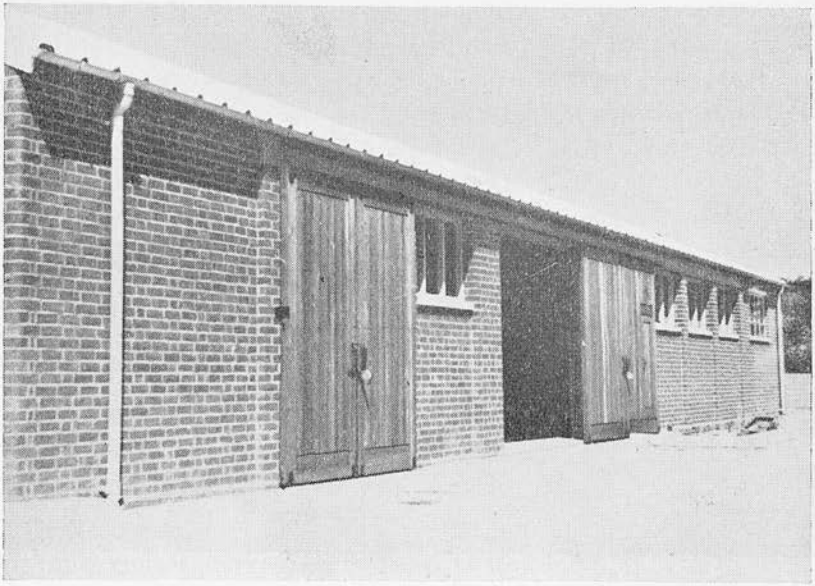

Fig. 3.

2. *Experimental animals.*

The majority of the Ayrshire cattle used in experiments at Compton have been bred at one or more of the three large dairy farms at the field station. These herds possess a long history of freedom from disease and the cattle are fully susceptible to experimental infection. Generally speaking heifers of breeding age have been employed in order to shorten the duration of the experiment and give speedier results. Recently, when it became necessary to assemble considerably more animals than the field station alone could provide, Dr. Gordon conceived the idea that heifer calves should be collected at birth direct from their dams on various Ayrshire farms in Scotland, blood tested to confirm freedom from infection and reared for the first three days of life on colostrum of known quality collected from Compton cows. This was accomplished by deep freezing the colostrum soon after collection some months previously. For transportation, cartons each containing a pint of colostrum were packed in suitable containers with dry ice. The colostrum was reconstituted at the field laboratory in Ayrshire and heated to 37° C. prior to

use. Subsequently the calves were fed on bulk milk from a non-reacting herd in the vicinity of the collecting centre. Within three weeks of birth the calves in batches of fifty were transferred by road to Compton — 400 miles distant.

3. *Experimental infection.*

In order to test a vaccine for immunising power, a known infection must be produced which closely resembles the naturally occuring disease. Various test doses have been tried but the most suitable appears to be about 150 million viable *Br. abortus* strain 544. This dose contained in a volume of 0.1 ml. is dropped directly into the conjunctival sac. In preparing the infecting suspension a standard dried culture is used, the inoculum is checked by plate counts and its virulence confirmed by guinea-pig inoculation. The procedure for testing a vaccine is shown in detail in Fig. 4. In a simple experiment, ten heifers would be vaccinated before service and another ten animals left as controls. The standard challenge dose would be applied to each individual, about the fifth month of the gestation period. Eventually, at calving or abortion, the amount of infection in each group would be determined by cultural and biological tests and the protective power of the vaccine thereby assessed. Invariably all the non-vaccinated controls become infected and with an efficient vaccine about 80 % protection can be expected in the vaccinated group. In this way, over a number of years the merits and disadvantages of a variety of living attenuated vaccines and non-viable antigens have been investigated; some of these results are summarised in Figs. 5, 6 and 7-complete details have already been published; Edwards, de Ropp and McLeod (1945), Edwards, McDiarmid, de Ropp and McLeod (1946), Mc Diarmid, (1949, 1950).

From these results, now adequately confirmed in the field, there can be little doubt that S. 19 remains the antigen of choice in Britain, although by no means the complete and perfect answer to the problem. Undoubtedly it possesses certain characteristics which place it above all other vaccines. It is comparatively easy to produce, is stable in character as shown by Mingle Manthei and Jasmin (1941) in the U.S.A. and Taylor and Mc Diarmid (1949) in Britain and confers adequate protection.

Although many of the problems relating to immunity have now been solved, the question of degree and duration of protection afforded by S. 19 still remains. In order to try and clarify this point a large scale experiment involving 534 heifers is now in progress at Compton; a plan of this work is shown in Fig. 8. It will be noticed that we have confined ourselves to vaccination prior to breeding age. The reason is that vaccination of adult cows may produce an adverse effect on the milk yield as shown by Holman and McDiarmid (1945) and it is also desirable that the reaction of the animal to the agglutination test should become negative as soon as possible after vaccination. This experiment commenced in 1949 and is due to terminate in 1956. Already, some results are available following the application of the first test dose and these show, quite clearly, that the immunity conferred by three vaccinations before breeding age is considerably better than that produced by the more commonly employed single or double vaccinations.

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THE METHOD EMPLOYED FOR TESTING Br. Abortus VACCINES IN CATTLE.

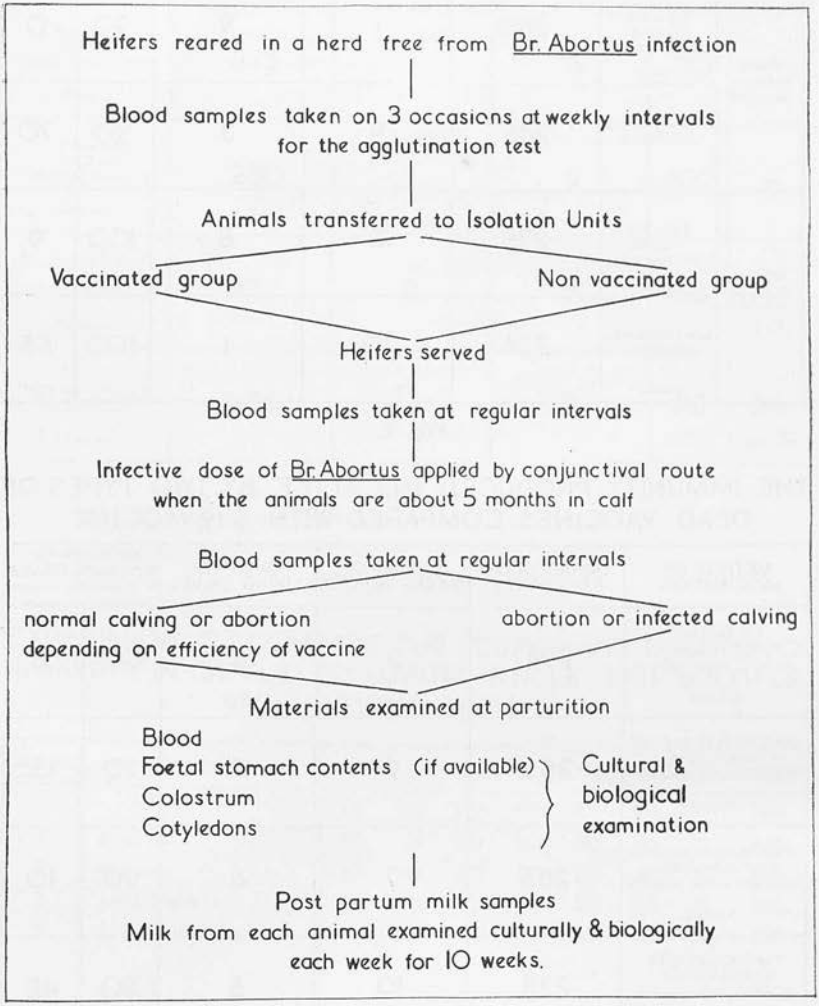

Fig. 4.

THE IMMUNITY PRODUCED IN CATTLE BY VACCINATION WITH S.19 LIVING VACCINE.

TEST DOSE.	METHOD OF VACCINATION.	DURATION OF PREGNANCY.	NUMBER OF HEIFERS INFECTED	NUMBER OF LIVING CALVES.	EXCRETION OF Br. Abortus IN THE MILK. No. of samples examined.	EXCRETION OF Br. Abortus IN THE MILK. No. of samples positive.
15 million Br. Abortus Strain 544	5·0 ml S.19 VACCINE SUBCUTANEOUSLY. 9 heifers	280	1	9	90	0
	NON VACCINATED CONTROLS 9 heifers	246	9	3	90	70
150 million Br. Abortus Strain 544	5·0 ml S.19 VACCINE SUBCUTANEOUSLY. 10 heifers.	275	2	8	100	9
	NON VACCINATED CONTROLS 10 heifers	224	10	1	100	55

Fig. 5.

THE IMMUNITY PRODUCED IN CATTLE BY TWO TYPES OF DEAD VACCINES COMPARED WITH S 19 VACCINE.

METHOD OF VACCINATION.	DURATION OF PREGNANCY.	NUMBER OF HEIFERS INFECTED.	NUMBER OF LIVING CALVES.	EXCRETION OF Br. Abortus IN THE MILK. No. of samples examined.	EXCRETION OF Br. Abortus IN THE MILK. No. of samples positive.
S 19 VACCINE. SUBCUTANEOUSLY. 60,000 million organisms. 10 heifers.	274	2	8	100	2
LANOLIN DEAD VACCINE. INTRAMUSCULARLY. 60,000 million organisms. 8 heifers	253	7	5	70	15
LANOLIN DEAD VACCINE. INTRAMUSCULARLY. 600,000 million organisms 9 heifers	265	7	6	90	10
FRACTION VACCINE INTRADERMALLY. 60,000 million organisms. 10 heifers	238	10	5	80	42
NON VACCINATED CONTROLS. 11 heifers.	248	10	5	110	37

Fig. 6.

THE IMMUNITY PRODUCED IN CATTLE BY DIFFERENT METHODS OF VACCINATION WITH S19 VACCINE.

METHOD OF VACCINATION.	DURATION OF PREGNANCY	NUMBER OF HEIFERS INFECTED	NUMBER OF LIVING CALVES	EXCRETION OF Br. Abortus IN THE MILK.	
				No. of samples examined	No. of samples positive.
5·0 ml. S.19 VACCINE SUBCUTANEOUSLY 8 heifers	275	1	6	70	—
0·2 ml. S.19 VACCINE INTRADERMALLY 10 heifers	280	1	9	100	—
1·0 ml. S.19 VACCINE INTRACAUDALLY 10 heifers	272	2	7	88	1 1·1%
NON VACCINATED CONTROLS 12 heifers	206	12	2	118	24 20·3%

Fig. 7.

EXPERIMENT TO COMPARE THE DEGREE AND DURATION OF IMMUNITY IN CATTLE FOLLOWING SINGLE AND MULTIPLE VACCINATIONS WITH S.19.

GROUPS	AGE AT VACCINATION	NUMBERS OF CATTLE TO BE TESTED AT EACH PREGNANCY							
		1st	2nd	3rd	4th	5th	6th	Spares	Total
1	6 months	10	10	20	20	20	20	5	105
2	18 months	10	10	20	20	20	20	5	105
3	6 and 12 months	10	10	10	20	20	20	15	105
4	6, 12 and 18 months	10	10	10	20	20	20	15	105
5	Controls								
	Fellows to those in groups 1 to 4	5	5	10	10	10	10	7	57
	Compton cattle	5	5	10	10	10	10	7	57
									534

Fig. 8.

Summary.

A description is given of the methods used at Compton for assessing the value of any given vaccine against brucellosis. A standard test dose is applied to vaccinated cattle of known origin together with suitable controls, at the fifth month of the gestation period. Subsequently the amount of infection in each group is determined by cultural and biological tests at parturition. In this way the immunising value of the vaccine can be accurately assessed. Attention is drawn to the large scale experiment, at present in progress, to determine the degree and duration of immunity conferred by single and multiple doses of S. 19 vaccine.

L'estimation de la valeur immunisante des vaccins antibrucelliques.

Résumé.

L'auteur décrit les méthodes utilisées à Compton pour vérifier la valeur de n'importe quel vaccin contre la brucellose. Une dose standardisée de Br. abortus est administrée à un groupe de vaches vaccinées dont l'origine est connue, ainsi qu'à un groupe de contrôle, au cinquième mois de la gestation. Ensuite, l'intensité de l'infection dans chacun des groupes est determinée au moyen d'épreuves biologiques et de culture au moment de la parturition. De cette façon, il est possible d'estimer avec précision la valeur immunisante de la souche en question.

L'auteur fait remarquer l'essai sur une grande échelle, couramment en état de progrès à Compton, qui a pour but la détermination du degré et de la durée de l'immunité conferée par une seule dose et par doses multiples de la souche 19.

Bestimmung der Immunisierungskraft von Brucella abortus-Vakzine beim Rindvieh.

Zusammenfassung.

Der Verf. beschreibt die in Compton angewandten Methoden zur Bestimmung der Immunisierungskraft von Vakzine gegen Brucella abortus. Alle Versuchstiere stammen aus brucellafreien Beständen. In einem einfachen Experiment werden 10 Färsen vor der ersten Deckung vakziniert, während 10 andere Färsen als Kontrolle dienen. Alle Färsen beider Gruppen werden im fünften Trächtigkeitsmonat mit einer Testdosis von Brucella abortus infiziert. Bei der Geburt oder bei Abort wird die Intensität der Infektion durch kulturelle und biologische Methoden bestimmt, und auf diese Weise ist es möglich genau das Immunisierungsvermögen der Vakzine zu bestimmen.

Zum Schluss erwähnt der Verf. eine, in Compton gross angelegte Untersuchung, die den Grad und die Dauer der Immunität nach einer oder mehrerer Vakzinationen mit Stamm 19 bestimmen soll.