RINDERPEST IMMUNITY IN CALVES

II. ACTIVE IMMUNIZATION*

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(With 1 Figure in the Text)

It has long been recognized that active immunization of calves against rinderpest may not always be satisfactory [review by Brown (1958*a*)].

This paper records the results of investigations to determine the serological response of calves possessing varying levels of maternally derived rinderpest antibody, to the inoculation of attenuated rinderpest viruses.

MATERIALS

Cattle. The majority of calves used were East African Shorthorn–Zebus, the progeny of dams which had been immunized 6–17 months before parturition by inoculation with lapinized rinderpest virus followed 2–4 weeks later by caprinized rinderpest virus. The calves were 2 days to 12 months old at the time of inoculation; all were weaned when 9 months old. Other calves aged from 1 day to 2 months were either Ayrshire, Friesian or crossbred European–Zebu, the progeny of susceptible dams. Rinderpest-susceptible cattle, aged 2 years, were crossbred European–Zebu. All cattle were reared and kept on rinderpest-free farms.

Rabbits. The rabbits were similar to those described in the previous paper (Brown, 1958*b*).

Lapinized rinderpest virus. As described in the previous paper (Brown, 1958*b*). Material of the sixteenth and eighteenth Kenya passages was used for the inoculation of calves and adults born of rinderpest-susceptible dams. The virus was in the form of a freeze-dried infected rabbit tissue suspension reconstituted and diluted with normal saline and inoculated in 2 ml. doses.

Caprinized rinderpest virus. The Kabete-Vom-Kabete strain of caprinized rinderpest virus (K.A.G.) at the 619th passage level was used. Aliquots of the virus were stored at $-25°$ C. as vacuum-sealed ampoules of freeze-dried infected goat spleen.

METHODS

Management of cattle. Calves from rinderpest-susceptible dams, aged from 1 to 68 days, and five adult cattle, aged 2 years, were each bled, inoculated subcutaneously with eighty cattle ID_{50} of lapinized rinderpest virus and again bled 21 days later. Serum was also obtained from the dams at the time the calves were inoculated. In attempts to immunize calves from immune dams, sixty-five animals

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were divided into thirteen groups of ages 2–6 days, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11 and 12 months. The calves were bled and each inoculated subcutaneously with 2 ml. of caprinized rinderpest virus that had been reconstituted and diluted with normal saline to give 100 cattle ID_{50} per ml. The morning rectal temperatures of the calves were recorded for 2–3 weeks after virus inoculation. All calves in which a temperature rise of 2° F. above the normal occurred for 3 or more days during the period 3–8 days following the inoculation of K.A.G. virus were considered to have shown a thermal reaction. Those calves which also showed signs of diarrhoea were noted. The calves were bled 21 days and 1 year (365 days) after inoculation. All the dams whose calves possessed no rinderpest antibodies in their sera before K.A.G. inoculation were bled 23–24 months after immunization and the sera screened for neutralizing antibodies.

Six calves from immune dams who were inoculated with K.A.G. virus when aged 3 days to 7 months but who apparently did not develop immunity as there were no serum antibodies 1 year later, were re-inoculated with 80 cattle ID_{50} of lapinized rinderpest virus 12–15 months after the K.A.G. inoculation.

Sera were stored for periods of up to 9 months at minus 25° C. and were titrated for rinderpest neutralizing antibodies using the method previously described (Brown, 1958*b*).

Data were analysed by standard statistical methods.

RESULTS

The active immunization of calves, the progeny of susceptible dams

The immune response of calves, the progeny of susceptible dams, and of adult cattle, following the inoculation of lapinized rinderpest virus, is given in Table 1. Differences between the titres of rinderpest neutralizing antibody, 21 days after inoculation, produced by calves aged from 1 day to 2 months at the time of inoculation and those of adult cattle were not significant ($t = 0{\cdot}7662$, $P > 0{\cdot}05$). None of the calves' dams possessed detectable rinderpest antibodies in their sera.

A preliminary examination of rinderpest neutralizing antibody production in very young calves (Brown, 1956) showed that calves 1 day old produced significant neutralizing antibody after inoculation.

Active immunization of calves, the progeny of immune dams

Pre-inoculation, 21 day and 1-year post-inoculation serum titres of rinderpest neutralizing antibodies and clinical reactions of calves, the progeny of immune dams, inoculated with caprinized rinderpest vaccine (K.A.G.) are recorded in Table 2.

All the dams of those calves which possessed no detectable antibody at the time of inoculation were shown to be immune.

Calves which possessed no detectable rinderpest antibodies 1 year after inoculation were considered not to have been actively immunized by the caprinized rinderpest virus. Thus, the serum titres of the calves 1-year post-inoculation indicated that no calf aged 3 months or less at the time of inoculation produced antibodies. On the other hand, all calves inoculated when aged 8 months or more

were actively immunized. Some of the calves aged from 4 to 7 months were immunized, others were not; a proportion of those which possessed maternally derived antibodies at the time of K.A.G. inoculation were also actively immunized.

All calves which had a pre-inoculation titre of 0·7 or less produced antibodies, whereas no calf did so if it had a pre-inoculation titre of 2·2 or greater. The response of calves with intermediate titres varied.

The 'amount' of antibody produced during the 21 days following inoculation was calculated by subtracting the pre-inoculation titre from the post-inoculation titre and, in the case of those calves in which antibodies were present before inoculation, adding 0·2 to the result. The 0·2 was added because this was approximately the amount by which the pre-inoculation titre would have fallen during 21 days, the half-life being about 37 days (Brown, 1958*b*).

Table 1. *The production of rinderpest antibodies by calves from susceptible dams and by adult cattle, following lapinized rinderpest virus inoculation*

Cattle no.	Age at inoculation	Serum titre* of rinderpest neutralizing antibodies: Pre-inoculation	Serum titre* of rinderpest neutralizing antibodies: 21 days post-inoculation
N 2	1 day	Nil	1·8
K 1	1 day	Nil	3·2
N 4	4 days	Nil	2·9
N 5	5 days	Nil	2·7
N 7	5 days	Nil	2·3
N 6	6 days	Nil	2·7
N 11	29 days	Nil	2·5
K 10	31 days	Nil	3·1
K 12	63 days	Nil	2·8
2974	2 years	Nil	2·4
2975	2 years	Nil	2·4
2979	2 years	Nil	2·4
2981	2 years	Nil	2·9
2982	2 years	Nil	3·1

* Expressed as the reciprocal of the logarithm of the 50% end-point dilution.

In those instances where calves were actively immunized following inoculation with caprinized rinderpest virus, it was found:

(*a*) There was a significant relationship between the pre-inoculation titre or age of calf at inoculation and the amount of antibody produced during the 21 days after inoculation ($r = -0{\cdot}7136$, $P < 0{\cdot}001$). Where the pre-inoculation titre was low or zero, the amount of antibody produced was large; conversely, where the pre-inoculation titre was high the amount of antibody produced was low.

(*b*) There was a significant relationship between the pre-inoculation titre or age of calf at inoculation and the 1-year post-inoculation titre ($r = -0{\cdot}5554$, $P < 0{\cdot}001$). In those calves which had little or no maternally derived antibody at the time of inoculation, the 1-year post-inoculation titres were higher than in those calves in which the pre-inoculation titres were high.

Table 2. *The response of calves aged from 2 days to 1 year, the progeny of immune dams, to caprinized rinderpest vaccine*

Age group	Calf no.	Age at inoculation in days	Post-inoculation thermal reaction	Pre-inoculation titre*	Post-inoculation titre	
					21 days	1 year
2–6 days	462	3	–	2·3	2·3	Nil
	463	3	–	2·9	3·1	Nil
	464	6	–	3·4	2·9	Nil
	466	2	–	2·8	2·6	Nil
	468	3	–	2·7	2·0	Nil
1 month (30 days)	457	29	–	2·7	2·0	Nil
	458	28	–	2·3	2·0	Nil
	459	28	–	2·8	2·8	Nil
	460	33	–	2·4	2·4	Nil
	461	28	–	2·5	2·2	Nil
2 months (61 days)	452	64	–	2·7	2·0	Nil
	453	61	–	3·0	2·3	Nil
	454	59	–	2·0	2·0	Nil
	455	59	–	1·4	1·5	Nil
	456	58	–	2·5	2·3	Nil
3 months (91 days)	447	89	–	2·4	1·8	Nil
	448	88	–	1·2	1·2	Nil
	449	91	–	2·6	1·9	Nil
	450	88	–	2·2	1·9	Nil
	451	87	–	1·8	1·2	Nil
4 months (122 days)	442	123	–	2·0	1·8	1·9
	443	123	–	1·9	1·8	Nil
	444	123	–	1·2	1·3	Nil
	445	122	–	1·7	1·1	Nil
	446	121	–	2·3	2·0	Nil
5 months (152 days)	437	155	–	1·1	1·2	Nil
	438	154	–	1·0	1·3	1·9
	439	153	–	1·0	1·7	2·3
	440	153	+	0·9	2·3	2·3
	441	152	–	1·6	1·3	1·8
6 months (182 days)	431	186	–	0·6	1·8	2·4
	432	181	+	0·5	2·6	2·2
	434	180	+	0·7	2·6	2·4
	435	182	–	0·3	2·0	1·4
	436	179	–	1·1	1·7	1·6
7 months (213 days)	426	210	+ +	< 0·3	2·8	2·5
	427	209	–	0·9	1·0	1·9
	428	208	–	0·9	1·0	Nil
	429	208	–	1·4	1·2	1·9
	430	207	+	0·6	2·0	2·4
8 months (243 days)	319	243	+	< 0·3	2·4	2·9
	417	246	+ +	< 0·3	2·5	2·4
	418	243	+	0·5	2·5	2·5
	419	244	+	< 0·3	2·5	1·8
	423	242	+	1·2	2·6	2·3

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Table 2 (*cont.*)

Age group	Calf no.	Age at inoculation in days	Post-inoculation thermal reaction	Pre-inoculation titre*	Post-inoculation titre	
					21 days	1 year
9 months	294	271	+	<0·3	2·7	2·0
(274 days)	295	273	+	0·3	2·8	2·5
	313	270	+	Nil	2·6	2·7
	314	276	+	Nil	2·8	2·4
	318	278	+	<0·3	2·6	2·4
10 months	288	313	+ +	<0·3	2·5	2·3
(304 days)	289	312	+	Nil	2·8	2·7
	290	310	+ +	Nil	3·4	2·8
	291	309	+	Nil	2·8	2·6
	292	309	+ +	<0·3	3·0	2·9
11 months	273	343	+ +	Nil	3·0	2·6
(334 days)	275	340	+ +	<0·3	3·3	2·9
	277	340	+	<0·3	3·3	2·4
	278	339	+	<0·3	3·2	2·2
	279	338	+	Nil	2·5	Died†
12 months	263	375	+ +	Nil	3·3	2·7
(365 days)	264	375	+ +	Nil	2·8	1·9
	269	359	+ +	Nil	2·9	2·5
	270	364	+	Nil	3·0	2·7
	271	363	+	Nil	3·4	2·7

* Expressed as the reciprocal of the logarithm of the 50 % end-point dilution.
† Died from acute rumenal tympany 11 months after inoculation.
\+ Thermal reaction.
\+ + Thermal reaction, together with diarrhoea following K.A.G. inoculation.
\− No thermal reaction following K.A.G. inoculation.

Table 3. *The mean pre-inoculation and* 1*-year post-inoculation serum titres of rinderpest neutralizing antibody and the 'amount'* of antibody produced during the* 21 *days after inoculation, of those calves, of various age groups, which were actively immunized following caprinized rinderpest virus inoculation*

Age group (months)	No. of observations	Mean titre†		
		Pre-inoculation	Antibody produced during 21 days after inoculation	1-year post-inoculation
12	5	Nil	3·1	2·5
11	5	0·2	3·0	2·5
10	5	0·1	2·9	2·7
9	5	0·2	2·7	2·4
8	5	0·5	2·2	2·4
7	4	0·8	1·2	2·2
6	5	0·6	1·7	2·0
5	4	1·1	0·8	2·1
4	1	1·8	0	1·9

* Post-inoculation titre minus pre-inoculation titre + 0·2 where antibody present before inoculation.
† Expressed as the reciprocal logarithm base_{10} of the 50 % neutralizing dilution.

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The mean pre-inoculation and 1-year post-inoculation titres of rinderpest antibody and the titre of antibody produced during the 21 days after inoculation, of these calves, of various age groups, which were actively immunized following caprinized rinderpest virus inoculation, are recorded in Table 3.

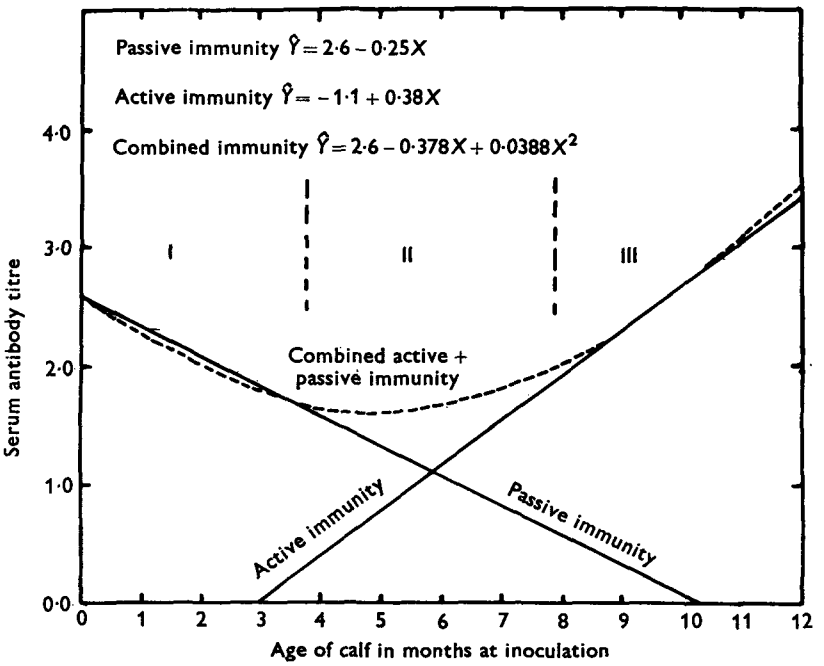

Fig. 1. The response of calves from rinderpest immune dams to caprinized rinderpest virus inoculation. Period I, no calves immunized; period II, some calves immunized; period III, all calves immunized. 'Active immunity' = antibody produced during 21 days following virus inoculation.

Table 4. *The immune response of calves, from immune dams, to lapinized rinderpest virus* 12–15 *months after the ineffective inoculation of lapinized rinderpest virus*

Calf no.	Age at time of caprinized rinderpest virus inoculation (months)	Age at time of lapinized rinderpest virus inoculation (months)	Serum titre* of rinderpest neutralizing antibodies		
			Pre-inoculation	4 days post-inoculation	21 days post-inoculation
428	7	20	Nil	Nil	2·9
437	5	17	Nil	Nil	2·8
443	4	17	Nil	Nil	2·5
447	3	17	Nil	Nil	2·6
457	1	16	Nil	Nil	2·6
462	3 days	15	Nil	Nil	2·6

* Expressed as the reciprocal of the logarithm of the 50 % neutralizing dilution.

Findings on maternally derived passive immunity and active immunity to rinderpest in calves from immune dams, are summarized diagrammatically in Fig. 1.

The immune response of calves, from immune dams, to the inoculation of lapinized rinderpest virus 12–15 months after the ineffective inoculation of caprinized rinderpest virus, is recorded in Table 4.

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DISCUSSION

Hitherto the serological response of very young calves to viable viral antigens has not been ascertained. We found that day-old calves responded to lapinized rinderpest virus just as well as adults. This result is comparable to that obtained with skin homographs, which induced vigorous reactions following grafting on to day-old calves (Billingham & Lampkin, 1957). *Trichomonas foetus*, *Brucella abortus* and *Salmonella dublin* antigens behaved differently (Kerr & Robertson, 1954; Kerr, 1956). We might suggest that apparent failures of very young calves to produce antibodies following the inoculation of bacterial or protozoal antigens could be attributed to weaknesses in the techniques used to measure the responses. However, with *Tr. foetus* antigen at least, this objection is not valid, for not only were calves of under the age of 1 month unable to produce agglutinins but three calves of 23 or less days of age acquired a tolerance to the antigen as shown by an impairment of their ability to respond to further antigenic stimulus (Kerr & Robertson, 1954). Development of tolerance necessarily implies an inability to produce antibodies.

We can only conclude, therefore, that a period of immunological immaturity occurs in calves after birth, but this immaturity only relates to some, not all antigens. The concept that very young calves may produce antibodies against one antigen but not against another is not revolutionary. Some skin homographs exchanged between very young chicks survived for long periods, but all chicks aged 2 weeks or more at the time of transplantation rapidly rejected the homographs (Cannon & Longmire, 1952). However, Wolfe & Dilks (1948) found that the proportion of chicks which produced precipitins to bovine serum did not reach 100 % until 5-week-old birds were used.

We are unable to say with absolute certainty that there is not a period of immunological immaturity in very young calves, so far as viable lapinized rinderpest virus antigen is concerned, because the calves were bled and the sera titrated 3 weeks after inoculation. If such a period of immaturity exists it must be 7 days or less because a period of 2 weeks is necessary for the rinderpest neutralizing antibody titre of serum to reach its maximum in adult cattle following lapinized rinderpest virus inoculation (MacOwan, 1956; Scott, 1956).

The finding that young calves of all ages between 1 and 63 days from rinderpest-susceptible dams produced neutralizing antibodies following the inoculation of lapinized rinderpest virus confirms Gillain's (1945) view that difficulties associated with immunizing some young calves against rinderpest are not due to an age factor.

Because of the difficulties associated with the immunization of young calves from immune dams against rinderpest, a special procedure has been adopted in areas of East Africa where compulsory rinderpest vaccination is enforced. All cattled presented for immunization are inoculated but only those animals which, in the opinion of the field officer, are more than 12 months old are branded to show that they have been immunized. Cattle less than 12 months old are not branded and, by law, must be re-presented for vaccination in the following year. This practical policy is fully justified by our experimental findings, because only calves

of 8 months of age or greater, if born of immune dams, were invariably immunized following the inoculation of caprinized rinderpest virus.

No calves of 3 months old or less from immune dams produced rinderpest antibodies, while results in calves aged between 4 and 7 months old varied. Some calves which possessed maternally derived rinderpest antibodies were actively immunized. The result depended upon the level of passive immunity.

Where calves were actively immunized the pre-inoculation titre influenced the titres found 21 days and 1 year after inoculation. Where the pre-inoculation titre was high the amount of actively produced antibody was low. Conversely, where little or no maternally derived antibody was present at the time of inoculation, the amount of antibody produced approached or equalled that produced by susceptible adult cattle. One year after K.A.G. virus inoculation, there were still significant differences between the titres of the calves inoculated at various ages. Those animals which had high titres 21 days after inoculation still had significantly higher titres than those in which the initial response was poor. However, the serum titres of actively produced antibody of this latter group had risen after the 21-day post-inoculation period; in many instances the 1-year post-inoculation titre was greater than the 21-day post-inoculation titre. Thus calves which possessed high pre-inoculation titres of maternally derived rinderpest antibodies, but which were actively immunized, produced antibodies at a slower rate than those calves which possessed little or no passive immunity before inoculation.

Many of the results obtained in the immunization of calves from immune mothers against rinderpest are similar to those obtained in the immunization against diphtheria of babies possessing maternally derived antitoxin. Calves could, under certain conditions, produce antibody when possessing a passive immunity, as could babies. Two levels of passive immunity were found to exist; all calves with titres below the lower level produced antibodies whereas none of those calves with titres above the higher limit did so. Also the amount of antibody produced and the rate of production depended upon the level of passive immunity existing at the time of inoculation. However, our finding that these differences in the amount of antibody produced in the 21 days following inoculation were still reflected in the serum titres 1 year after inoculation differs somewhat from results obtained from work on diphtheria immunization. Greenberg & Fleming (1951) reported that 1 year after inoculation of diphtheria toxoid there was no significant difference between the average serum antitoxin titres of babies who lacked detectable diphtheria antitoxin at the time of inoculation and those who possessed it.

The finding that all calves which possessed maternally derived rinderpest antibody to a titre of 0·7 or less produced antibody following K.A.G. inoculation is similar to results obtained by Scott (1956) in a different field using a similar rabbit neutralization test. He investigated the effect of inoculating bovine rinderpest virus into cattle which previously had been actively immunized by the same virus strain. All cattle in which the antibody titre had fallen to 1·0 or less produced antibody following the second inoculation of virus.

Some of the calves from immune dams inoculated with K.A.G. virus produced antibodies without showing thermal reactions, thus confirming the finding of

Rabagliati (1924). This finding suggests that Milne's (1956) view that maternally derived rinderpest immunity in calves from immune dams may last as long as 19 months is probably incorrect. In his experiment a calf from an immune dam was twice inoculated with K.A.G. virus, the second time being when it was 19 months old. No thermal reaction occurred after either inoculation. Therefore, concluded Milne, the maternally derived immunity was still present 19 months after birth. However, it seems probable that the calf was actively immunized when first inoculated without showing a thermal reaction.

Contrary to the findings of Milne (1956) we found that all calves which showed a thermal reaction following K.A.G. virus inoculation produced antibodies and were still immune 1 year later.

It is of interest to note that in our experiments all calves which showed signs of diarrhoea following caprinized rinderpest virus inoculation possessed little or no passive immunity.

Those calves which lacked rinderpest neutralizing antibodies in their sera 12–15 months after K.A.G. virus inoculation and which were then inoculated with lapinized rinderpest virus did not possess rinderpest antibodies in their sera 4 days after the latter inoculation. This indicated that in addition to the failure of the K.A.G. virus inoculation to stimulate the production of an active immunity, the antibody mechanism was not sensitized to rinderpest antigen, as Scott (1956) found that in cattle, where a secondary response to rinderpest antigen occurred, an increase in antibody titre was demonstrable 4 days after the second inoculation. Our results differ from those found in human infants (Osborn, Dancis & Julia, 1952) and lambs (Barr, Glenny & Howie, 1953) from diphtheria-immune mothers. In some instances where the maternally derived immunity was considerable, although there was no active production of antitoxin following diphtheria toxoid inoculation, the antibody-forming mechanism was sensitized as shown by typical secondary responses following re-inoculation of the antigen.

SUMMARY

Calves which were the progeny of rinderpest-susceptible dams were inoculated with lapinized rinderpest virus when 1 day to 2 months old. Their serological response, as measured by neutralizing antibody titre 21 days later, did not differ from that of adult susceptible cattle.

In calves born of rinderpest-immune cows, the serological response to caprinized rinderpest virus depended upon the level of maternally derived antibody. There were two critical levels, one below which all calves produced antibodies (serum titre of 0·7 or less) and another above which no antibody was formed (serum titre of 2·2 or more). Thus all animals aged 8 months or more at the time of inoculation were actively immunized but no calf aged 3 months or less reacted in this manner.

The response of calves with maternally derived antibody titres between 0·9 and 2·0 varied. There was a significant inverse relationship between the pre-inoculation titre and the amount of antibody formed during the following 3 weeks.

A similar inverse relationship was shown between the pre-inoculation titre and the rate of production of antibody and its titre 1 year following inoculation.

Calves which possessed colostral antibody, and which were actively immunized by caprinized virus inoculation, did not necessarily show the usual clinical reaction. When such animals failed to become actively immunized there was no sensitization of the antibody-forming mechanism, a fact demonstrated by the lack of an anamnestic response to subsequent exposure to rinderpest virus antigen.

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