RINDERPEST IMMUNITY IN CALVES

I. THE ACQUISITION AND PERSISTENCE OF MATERNALLY DERIVED ANTIBODY*

BY R. D. BROWN

East African Veterinary Research Organization, Muguga, Kenya

(With 1 Figure in the Text)

INTRODUCTION

Mammalian mothers possessing an active immunity against some diseases confer upon their progeny a passive immunity which may afford protection until the young animal is actively immunized. Since the first description of this phenomenon in lambs by Bollinger (1877), the transmission of maternal antibodies to the young has been investigated in many animal species. Reviews of the literature include those of Ratner, Jackson & Gruehl (1927), McGirr (1947) and Brambell, Hemmings & Henderson (1951).

Montgomery (1915) reported that calves cannot always be actively immunized against rinderpest; since then attempts to determine the duration of persistence of maternally derived immunity in calves have yielded a wide range of results (review by Brown, 1958*a*).

The problem has therefore been re-investigated, using a rabbit test for rinderpest neutralizing antibody, and the results are reported in this communication.

MATERIALS

Cattle. With few exceptions all calves were East African Shorthorn–Zebus born of dams which had been immunized against rinderpest by inoculation, 6–17 months before parturition, with lapinized rinderpest virus followed 2–4 weeks later with caprinized rinderpest virus (K.A.G.). Calf no. 1592 was a crossbred European–Zebu from a dam inoculated with K.A.G. virus 5 months before parturition, followed 3 weeks later by challenge with bovine rinderpest virus. Calf 1722 was of the same breed, born of a cow immunized with K.A.G. virus 10 months before parturition. All calves were weaned when 9 months old, except nos. 1592 and 1722 which were weaned when 6 months old. Two Friesian cows were immunized to provide immune milk for feeding to susceptible calves which had passed the neonatal period. All cattle were kept on rinderpest-free farms.

Rabbits. The rabbits were of the Albino, Chinchilla, Belgian Hare and Rex breeds or their crosses. All were equally susceptible to lapinized rinderpest virus.

Lapinized rinderpest virus. The Nakamura III strain of lapinized rinderpest virus in its 15th, 17th and 18th Kenya passages was used for neutralization tests, and the 18th passage for the active immunization of two dams. On arrival from China in 1949 the virus strain had undergone 795 serial rabbit passages. Aliquots of virus

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were stored in ampoules at minus 25° C. as a freeze-dried vacuum-sealed 25% infected rabbit tissue suspension (Brotherston, 1951).

METHODS

Management of cattle. Seven calves were used to determine the mode of transmission of maternal rinderpest antibodies and their half-life in calves. With the exception of calf 1592, which was born unobserved, they were separated from their dams immediately after birth and bled before being allowed to suckle. At the same time blood and colostrum samples were obtained from the cows. The calves were then allowed to suckle, bled 30–48 hr. later, and subsequently at 30-day intervals for periods of up to 1 year. Samples of milk were obtained from some of the dams during the period of 2 weeks after parturition.

Sixty-five calves from immune dams were divided into thirteen groups aged 2–6 days, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11 and 12 months respectively, and serum samples obtained from all of them.

To determine whether calves of more than 2 days old could absorb rinderpest neutralizing antibodies from the intestinal tract, colostrum samples were obtained 2–3 hr. after calving from two rinderpest-susceptible cattle. One day after calving the two cows and their calves were bled, and the cows both inoculated subcutaneously with eighty cattle ID_{50} of lapinized rinderpest virus. Serum samples were obtained from all four animals, 7, 14, 21 and 42 days later. Milk samples were collected from the two cows at intervals during the following 21 days, and again at 42 days after virus inoculation. One gallon of its dams milk was fed daily to each calf.

Sera were stored in screw-capped bottles at minus 25° C. for periods of up to 15 months. Standard immune serum was stored at 4° C. after freeze-drying in vacuum-sealed ampoules.

Colostrum and milk wheys. 0·1 g. of rennet powder* was added to about 200 ml. of colostrum or milk, pre-warmed to 37° C. and the mixtures left on the bench overnight to clot. The wheys were then decanted, centrifuged and stored in screw-capped bottles at minus 25° C. for periods of up to 15 months.

Rinderpest neutralizing antibodies. Sera and wheys were inactivated at 56° C. for 30 min., immediately before dilution. Fivefold dilutions of the serum or whey under test were prepared using Lemco broth (Oxo Ltd.), pH 7·2–7·4 as a diluent. The virus was reconstituted by suspension of the contents of 6 ampoules in the original volume of distilled water. This suspension was diluted 1/10 with broth and and centrifuged for 3 min. at 1500 r.p.m. to remove large particles of tissue. The supernatant was further diluted with broth so that each ml. contained approximately 200 rabbit ID_{50} per ml. after incubation for 1 hr. at 37° C. Equal volumes of virus and three or four serum dilutions were mixed and incubated for 1 hr. at 37° C. Serum-virus mixtures were inoculated intravenously into rabbits in 1 ml. doses, five rabbits being used per dilution. Between incubation and inoculation the bottles of serum-virus mixtures were kept on ice, avoiding exposure to direct sunlight.

* 'Ha-La' Brand. Manufactured by Chr. Hansens Laboratory Ltd., Reading, England.

Controls in each test included a virus titration, a known non-immune serum-virus mixture and the titration of threefold dilutions of a standard immune serum. The latter made possible the standardization of results obtained in different tests. The stock serum had a titre of $10^{2\cdot0}$ against $10^{1\cdot9}$ to $10^{2\cdot1}$ rabbit ID_{50} of virus.

Five days after inoculation the rabbits were killed and examined for lesions of lapinized rinderpest virus infection (Fukusho & Nakamura, 1940). Rabbits dying before the fifth day after inoculation were likewise examined, and if lesions were present the animal was included in the titration. A few animals dying without lesions were excluded. The dilution of serum or whey at which 50% of the inoculated rabbits were infected was calculated using Thompson's method (1947) and the results expressed as the reciprocal of the logarithm to the base 10.

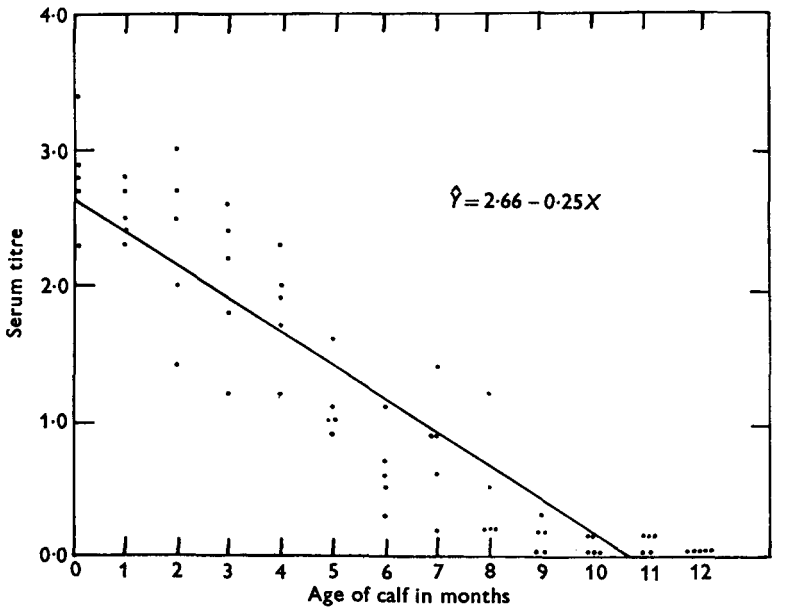

Fig. 1. The duration of naturally acquired passive rinderpest immunity calculated from the titres of sixty-five calves.

Where sera were expected to have either little or no detectable neutralizing antibody, they were tested at a dilution of 1/2; if all rabbits were infected the titre was expressed as 'nil'; if three or four rabbits were infected the serum titre was expressed as $< 0{\cdot}3$ and if only one or two reacted a normal titration was performed. Where calves of higher age-groups showed no antibody, the sera of their mothers were tested at a dilution of 1/20, three rabbits only being inoculated.

Data were analysed by standard statistical methods.

RESULTS

The transfer of maternally derived rinderpest antibody and its half-life in the calf

The rinderpest neutralizing antibody titres of the sera of thirteen groups of calves born of immune dams and aged 2–6 days up to 12 months are recorded diagrammatically in Fig. 1. These titres are also recorded as the pre-inoculation titres in

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Table 2, in the second of these papers (Brown, 1958*c*). The dams of calves which possessed no antibodies were shown to be immune.

Titres of the sera of seven calves from immune dams, before and at various periods after suckling, are given in Table 1, together with the dams' sera at calving

Table 1. *Rinderpest neutralizing antibody titres* of the sera of calves, the progeny of immune dams, at various periods after birth, together with those of their dams' sera, colostral and milk wheys*

Dam no. ...	1131	199	221	212	240	219	203
Calf no. ...	1592	1722	420	421	422	425	433
Titres of							
Dams serum at calving	2·1	2·0	2·6	2·6	2·2	2·4	2·6
Colostral whey	Not available	3·3	3·4	3·2	3·3	3·6	3·0
Milk whey after calving							
30–48 hr.	—	2·1	—	—	1·2	—	—
1 week	—	1·3	—	—	1·6	—	—
2 weeks	—	0·6	—	—	1·0	—	—
Serum of calf							
Before suckling	Not available	Nil	Nil	Nil	Nil	Nil	Nil
30–48 hr. after suckling	2·8	3·0	2·9	2·3	2·7	3·0	3·1
1 month of age	2·7	2·4	—	—	—	—	2·3
2 months	2·4	—	—	—	—	2·5	—
3 months	1·9	2·0	1·5	2·1	—	—	—
4 months	1·6	—	—	—	1·3	—	1·6
5 months	1·9	—	—	—	—	1·3	—
6 months	1·8	1·2	0·8	1·2	—	—	—
7 months	1·2	—	—	—	—	—	0·6
8 months	0·7	0·6	< 0·3	—	< 0·3	0·7	< 0·3
9 months	< 0·3	—	—	< 0·3	—	—	—
10 months	< 0·3	< 0·3	< 0·3	—	< 0·3	—	Nil
11 months	—	Nil	< 0·3	< 0·3	Nil	< 0·3	—
12 months	—	—	—	Nil	—	< 0·3	—

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

Table 2. *Relationships between the rinderpest antibody titres of the dam's serum at calving, the colostral whey and the calf's serum after suckling*

	Calf no.						
Ratio	420	421	422	425	433	1722	Mean
Colostral whey/dam's serum	6·3	4·0	12·6	15·8	2·5	20·0	7·2
Calf's serum/colostral whey	0·32	0·13	0·25	0·25	1·26	0·50	0·45
Calf's serum/dam's serum	2·0	0·5	3·2	4·0	3·2	10·0	3·8

and their colostral and milk wheys. No calf serum taken before suckling contained detectable antibodies.

The titres of the colostral wheys were significantly greater than those of the sera of the calves after suckling ($t = 3{\cdot}424$, $P < 0{\cdot}01$), the latter being in turn higher than those of the dams ($t = 2{\cdot}530$, $P < 0{\cdot}05$).

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Relationships between the titres of the colostral whey, the calf's serum after suckling and the dam's serum are recorded in Table 2. No significant relationship was found between the titre of the colostral whey and the dam's serum at calving ($F = 0{\cdot}03$, $P > 0{\cdot}05$), the colostral whey and the calf's serum after suckling ($F = 0{\cdot}09$, $P > 0{\cdot}05$) or the dam's serum at calving and the calf's serum after suckling ($F = 1{\cdot}49$, $P > 0{\cdot}05$).

Maternally derived rinderpest antibody in the sera of calves declined exponentially with time and the regression was significant (Table 3). When individual regression lines for seven calves were compared with the composite line for 65 calves, differences were not significant.

The half-life of maternally derived antibody and its extinction point was calculated from individual regression lines. The mean half-life was 36·7 days and the extinction point 10·9 months (1 month = 30 days). The extinction point was the age at which it was calculated that the serum titre would have fallen to 0·0.

Table 3. *Decline of titre with time, half-life and extinction point of maternally derived passive rinderpest antibody in calves*

Calf no.	Regression line formula	F	Half-life antibody in days	Extinction point in months
65 calves	$\hat{Y} = 2{\cdot}7 - 0{\cdot}25X$	338·24	36	10·6
1592	$\hat{Y} = 2{\cdot}9 - 0{\cdot}26X$	139·25	34·5	11·2
1722	$\hat{Y} = 2{\cdot}8 - 0{\cdot}26X$	384·00	34·5	10·8
420	$\hat{Y} = 2{\cdot}5 - 0{\cdot}23X$	29·69	39	10·8
421	$\hat{Y} = 2{\cdot}5 - 0{\cdot}21X$	158·33	42·9	11·7
422	$\hat{Y} = 2{\cdot}5 - 0{\cdot}25X$	79·00	37·5	10·4
425	$\hat{Y} = 2{\cdot}8 - 0{\cdot}23X$	62·80	39	12·2
433	$\hat{Y} = 2{\cdot}8 - 0{\cdot}30X$	121·00	30	9·3
		Mean	36·7	10·9

The effect of feeding milk containing rinderpest neutralizing antibodies to calves which had passed the neonatal period

The serum titres of two cows and their calves, of colostral and milk wheys taken before and at various periods after inoculation of the dams with lapinized rinderpest virus are given in Table 4.

The colostral wheys of both dams lacked neutralizing antibodies. Rinderpest antibodies were present in milk wheys at 7 days but not at 4 days after the inoculation of lapinized rinderpest virus. Although the two calves each ingested daily 1 gallon of milk which contained rinderpest antibodies, between the ages of 8 and 43 days, no rinderpest antibodies were detected in their sera, thereby indicating that absorption of antibody from the alimentary tract did not occur.

DISCUSSION

Antibodies against bacterial, protozoal, rickettsial antigens and some bacterial toxins have been found to be transmitted from immune cows to their calves via the colostrum. Recently Schneider (1955) showed that the route of transfer of

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foot-and-mouth disease antibodies was the same. Similarly, maternally derived rinderpest antibodies were transmitted to the calf by the colostrum, since calves born of immune dams lacked antibodies at birth but possessed them to a high titre 30–48 hr. after suckling. This finding gives support to previous assumptions that rinderpest antibodies were transmitted through the colostrum, and refutes the suggestion of Rabagliati (1924) and Gillain (1944) that calves may possess rinderpest antibodies at birth.

The statement of Hale, Walker, Maurer, Baker & Jenkins (1946) that 'no immunity is conferred to calves born of (rinderpest) vaccinated animals' is clearly incorrect as a generalization. In our experiments all calves born of immunized

Table 4. *Failure of calves to absorb milk antibody after the neonatal period*

Time	Rinderpest neutralizing antibody titre* of: Dam's serum K. 20	Dam's serum K. 21	Dam's colostral or milk whey K. 20	Dam's colostral or milk whey K. 21	Calf's serum K. 20	Calf's serum K. 21
3 hr. after calving	—	—	Nil (Colostrum)	Nil (Colostrum)	—	—
1 day after calving. Dam inoculated with lapinized rinderpest virus	Nil	Nil	Nil (Milk)	Nil (Milk)	Nil	Nil
4 days after inoculation	—	—	Nil (Milk)	Nil (Milk)	Nil	Nil
7 days after inoculation	—	—	0·9 (Milk)	0·5 (Milk)	Nil	Nil
14 days after inoculation	—	—	0·9 (Milk)	0·9 (Milk)	Nil	Nil
21 days after inoculation	2·2	2·6	—	—	Nil	Nil
42 days after inoculation	—	—	0·8 (Milk)	0·9 (Milk)	Nil	Nil

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

dams acquired serum antibodies following ingestion of colostrum. The serum titre of calf no. 1592 after suckling, born of a dam immunized during pregnancy, did not differ from those of calves born of dams immunized before pregnancy. This finding conflicts with the view of Rabagliati (1924), who considered that calves born of cows immunized after conception possessed a greater passive immunity than those of dams immunized before conception.

Excretion of antibodies by the bovine mammary gland did not end when all the colostrum had been ingested by the calf; 2 weeks after calving, rinderpest neutralizing antibodies were present in the milk of immune dams, but to a titre of about 2 logs lower than that of the colostrum at the time of calving.

After newborn calves ingested colostrum the antibody titre in the serum declined linearly with time. This is similar to results obtained previously with diphtheria antitoxin in babies (Barr, Glenny & Randall, 1949) and lambs (Barr, Glenny &

Howie, 1953), and with lamb dysentery antitoxin in lambs (Mason, Dalling & Gordon, 1930). The mean extinction point of maternally derived rinderpest neutralizing antibodies in calves was 10·9 months. This period approximates to that reported for diphtheria antitoxin in babies (Neill, Gaspari, Richardson & Sugg, 1932; Barr *et al.* 1949) but exceeds that found, for example, with maternally derived *Brucella abortus* agglutinating antibodies in calves (McAlpine & Rettger, 1925; Thorp & Graham, 1933; McDiarmid, 1946), *Salmonella abortus-equi* agglutinating antibodies in foals (Bruner, Edwards & Doll, 1948) and *Corynebacterium diphtheriae* agglutinating antibodies in human infants (Neill *et al.* 1932).

The mean half-life of maternally derived rinderpest antibody was 36·7 days. This figure is similar to results obtained by other workers for diphtheria antitoxin in lambs (Barr *et al.* 1953) and human infants (Barr *et al.* 1949). The half-life of rinderpest antibody in calves was calculated without reference to the dilution of antibody by the growth of the calves. Attempts to apply the formula of Wiener (1951) for calculating the half-life of maternally derived antibody, while allowing for growth and increasing blood volume, gave unsatisfactory results as the variation was markedly increased.

We found no evidence to support the claim of Campbell, Sarwar & Petersen (1957) that absorption of intact antibody from the gastro-intestinal tract occurs after the neonatal period. Rinderpest antibodies were not detected at any time in the sera of two calves which, for a period of 5 weeks, consumed milk containing these antibodies. We realize that the number of calves used in the experiment was small. However, our findings received added support from the results of the experiments on the duration of maternally derived rinderpest antibodies in calves. The serum titres declined linearly on time, which suggested that reinforcement of the passive immunity was not occurring although the calves were ingesting milk for periods of 6–9 months after birth. Nor does the claim of Campbell *et al.* accord with the evidence of Comline, Roberts & Titchen (1951) and Hansen & Phillips (1947), who reported that absorption of antibody and gamma globulin from the gut of calves only occurred during the first few hours of life.

SUMMARY

Rinderpest neutralizing antibody was found to be transferred from the immune dam to the calf via the colostrum. No antibodies were detected in the sera of calves before suckling. The colostrum of immune cows contained rinderpest antibodies to a higher titre than that of the serum; 30–48 hr. after the ingestion of such colostra, newborn calves possessed high antibody levels in their sera, levels greater than those of their dams' sera but less than those of the colostrum ingested.

After the neonatal period the serum titres of calves declined linearly. The mean half-life of maternally derived rinderpest antibody in calves was 36·7 days and the extinction point 10·9 months.

Two young calves, from susceptible dams, which each ingested daily for 5 weeks 1 gallon of milk containing rinderpest antibodies failed to show evidence of their absorption from the intestinal tract.

REFERENCES

Barr, M., Glenny, A. T. & Howie, J. W. (1953). *J. Path. Bact.* **65**, 155.

Barr, M., Glenny, A. T. & Randall, K. J. (1949). *Lancet*, ii, 325.

Bollinger, O. (1877). *Samml. klin. Vortr.* no. 116. Cited by Edsall, G. (1956), in *Ann. N.Y. Acad. Sci.* **66**, 32.

Brambell, F. W. R., Hemimngs, W. A. & Henderson, M. (1951). *Antibodies and Embryos.* London: Athlone Press.

Brotherston, J. G. (1951). *J. comp. Path.* **61**, 263.

Brown, R. D. (1958*a*). *Bull. epiz. Dis. Afr.* **6**, 127.

Brown, R. D. (1958*c*). *J. Hyg., Camb.*, **56**, 435.

Bruner, D. W., Edwards, P. R. & Doll, E. R. (1948). *Cornell Vet.* **38**, 363.

Campbell, B., Sarwar, M. & Petersen, W. E. (1957). *Science*, **125**, 932.

Comline, R. S., Roberts, H. E. & Titchen, D. A. (1951). *Nature, Lond.*, **167**, 561.

Fukusho, K. & Nakamura, J. (1940). *Jap. J. vet. Sci.* **2**, 75.

Gillain, J. (1944). *Immunite congenitale et virus peste bovine adapte sur chevre*, pp. 3. Leopoldville, Congo Belge. Govt. General Service Veterinaire. fcp. Mimeographed.

Hale, M. W., Walker, R. V. L., Maurer, F. D., Baker, J. A. & Jenkins, D. L. (1946). *Amer. J. vet. Res.*, **7**, 212.

Hansen, R. G. & Phillips, P. H. (1947). *J. biol. Chem.* **171**, 223.

Mason, J. H., Dalling, T. & Gordon, W. S. (1930). *J. Path. Bact.* **33**, 783.

McAlpine, J. G. & Rettger, L. F. (1925). *J. Immunol.* **10**, 811.

McDiarmid, A. (1946). *Vet. Rec.* **58**, 146.

McGirr, J. L. (1947). *Vet. J.* **103**, 345.

Montgomery, R. E. (1915). Quoted by Stordy, R. J. (1916), in *Rep. Dep. Agric. B.E.A.* for 1915–16, p. 66.

Neill, J. M., Gaspari, E. L., Richardson, L. V. & Sugg, J. Y. (1932). *J. Immunol.* **22**, 117.

Rabagliati, D. S. (1924). *J. comp. Path.* **37**, 1.

Ratner, B., Jackson, H. C. & Gruehl, H. L. (1927). *J. Immunol.* **14**, 249.

Schneider, B. (1955). *Mh. Tierheilk.* **7**, 137. Abstracted in *Vet. Bull., Weybridge*, **26**, Abstr. no. 1227.

Thompson, W. R. (1947). *Bact. Rev.* **11**, 115.

Thorp, F. & Graham, R. (1933). *J. Amer. vet. med. Ass.* **82**, 871.

Wiener, A. S. (1951). *J. exp. Med.* **94**, 213.

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