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The effect of colostrum-derived antibody on louping-ill virus infection in lambs

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SUMMARY

The influence of colostrum-derived antibody to louping-ill virus on the course of experimental infection was investigated in lambs. Lambs that had high titres of antibody were refractory to infection. Lambs that had low titres of antibody did not develop a viraemia but either showed an antibody reaction or were sensitized as judged by the immune response, which was typical of an anamnestic response, after rechallenge. Animals that had no antibody 34–20 days before challenge had either no or very slight viraemia, but did develop an antibody response with titres as high as those of control lambs by day 21. Lambs that had been negative for longer periods responded in a similar fashion to controls.

These findings are discussed in relation to the occurrence of disease in lambs kept in louping-ill endemic areas. It is concluded that in such areas infections of lambs are likely to be of minor importance as a cause of mortality and of little epidemiological significance.

INTRODUCTION

In louping-ill endemic areas many lambs are born to immune dams and receive colostral antibody to the virus (Williams & Thorburn, 1961; Brotherston, Bannatyne, Mathieson & Nicholson, 1971). Such antibody is considered to be of major importance in protecting lambs from louping-ill (Wilson & Gordon, 1948; Williams & Thorburn, 1961; Smith *et al.* 1964; Smith, 1969). However, this aspect of infection has not been examined critically either in relation to the protection of lambs from disease or in the suppression of viraemia.

The present paper describes the course of infection in lambs which received colostral antibody and were subsequently challenged subcutaneously with virus.

MATERIALS AND METHODS

Laboratory procedures

Antibody to louping-ill virus was assayed using the haemagglutination inhibition (HI) test. The processing of sera for the test and treatment with 2-mercaptoethanol (2-ME) has been described previously (Reid & Doherty, 1971*a*). Challenge inocula were prepared from mouse brains infected with the SB/526 strain of virus (Reid & Doherty, 1971*a*). Each time lambs were challenged the inoculum was assayed for plaque forming units (p.f.u.) using the IB RS2 clone 60 pig kidney

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cell line (Reid & Doherty, 1971*b*). Infectivity of blood was also assayed using the plaque method. Heparinized blood was collected and 2 aliquots of each plasma stored at −70° C. until tested. One of these aliquots was thawed and tested for p.f.u. If the specimen gave a confluent cytopathic effect the duplicate aliquot was tested at a dilution which was estimated to give between 10 and 100 plaques per culture. All assays of infectivity were made by inoculating 0·2 ml. on to each of five replicate cultures.

Experimental animals and procedures

The lambs were the progeny of ewes that had been immunized with an inactivated cell culture vaccine (Brotherston & Boyce, 1969, 1970) and were selected from groups in which the decline of colostral antibody was being followed (Brotherston, personal communication). Throughout the period of investigation all animals were maintained on the Institute farms which are known to be free of *Ixodes ricinus*.

To determine the titre of colostral antibody that would modify infection an initial experiment was performed using 12 lambs aged 5–9 weeks which possessed a range of antibody titres. Four of the lambs had HI antibody titres of $\geqslant$ 1/160, four had titres between 1/10 and 1/40 and four, which had been found to have a titre of 1/20 within 10 days of birth, had no detectable antibody when challenged. Each lamb was inoculated subcutaneously (s.c.) with $10^{7\cdot08}$ p.f.u. and the reaction to challenge was assessed on the basis of the HI antibody response. Blood for serum separation was collected on the 6th, 10th, 13th, and 24th days after inoculation.

Following this initial investigation a further experiment was carried out. Twenty lambs aged 4–5 months which received colostral antibody were selected on the basis of their HI antibody status and were divided into the following four groups of five lambs; group 1, antibody present at a titre of 1/20; group 2, antibody became undetectable 34–20 days before challenge; group 3, antibody became undetectable 62–48 days before challenge; group 4, antibody became undetectable 90–76 days before challenge. A further five lambs which were the progeny of unvaccinated ewes served as controls (group 5). Plasma for virus assay was collected from each lamb 24 h. after inoculation and for the following 8 days. Sera were collected before inoculation, on the day of challenge and on alternate days for the first ten days as well as on days 14, 21 and 42. Additional serum samples were collected from groups 1 and 5 on days 71, 139 and 186.

The lambs in group 1 were again inoculated with virus 187 days after initial challenge. Plasma for virus isolation and serum was collected daily for the first 9 days after inoculation and serum only on days 14, 21, 28 and 35.

For both the initial challenge and for rechallenge the SB/526 strain was inoculated s.c. and it was calculated that on each occasion each lamb received $10^{7\cdot57}$ p.f.u.

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Table 1. *Degree of viraemia detected in lambs that received colostral antibody and were challenged with louping-ill virus*

Group	Lamb no.	Day after inoculation 1	2	3	4	5	6
(2) Negative for HI antibody 34–20 days before challenge	C/147	N	N	N	N	N	N
	149	N	N	N	N	N	N
	151	N	N	0·18	0·53	0·26	0·62
	199	N	N	N	N	N	N
	201	N	N	N	0·64	N	1·30
(3) Negative for HI antibody 62–48 days before challenge	C/143	0·30*	2·34	3·48	5·32	2·89	N
	157	1·60	0·42	1·51	3·85	2·60	N
	158	N	N	1·74	3·81	4·11	1·64
	206	1·30	0·01	0·01	N	N	N
	210	1·78	1·46	2·78	4·60	2·81	N
(4) Negative for HI antibody 90–76 days before challenge	C/185	0·51	2·26	3·04	4·53	2·42	N
	190	1·34	2·45	4·26	4·93	2·04	N
	193	0·30	2·28	3·56	4·74	1·82	N
	213	0·78	2·46	4·34	5·30	3·08	N
	224	N	0·26	0·76	1·88	N	N
(5) No colostral HI antibody received	C/196	0·51	2·49	3·00	4·28	2·83	N
	197	1·78	2·01	2·86	4·28	2·76	N
	198	1·90	1·90	2·15	2·60	1·78	N
	226	1·45	2·93	4·04	4·56	N	N
	291	N	N	0·81	2·32	2·11	N

N = no virus detected.
* Log_{10} p.f.u./0·2 ml.

RESULTS

Initial experiment

None of the lambs in the first experiment developed clinical signs of louping-ill virus infection. The antibody titres in the lambs that had titres of $\geqslant$ 1/160 when challenged declined throughout the period of observation. The antibody titre in serum from the single lamb with an initial titre of 1/40 declined to 1/20 by day 6, but was 1/40 and 1/80 on days 10 and 24 respectively. All the lambs with initial antibody titres of between 1/10 and 1/20 experienced an antibody response and on day 24 after inoculation their titres ranged from 1/20 to 1/80. The lambs which were negative when challenged all developed HI antibody by day 6, the maximum levels of between 1/20 and 1/160 being reached by day 13.

Second experiment

Clinical signs were observed in two of the 25 lambs inoculated with virus. One animal in group 3 became ataxic on day 10 and died on day 11 and in group 4 one animal died on day 9 having developed signs of neurological dysfunction on day 8. None of the other lambs showed obvious signs of ill health.

No virus was detected in the plasmas of animals in group 1 and only low titres

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Table 2. *The reciprocal haemagglutination inhibiting antibody titres in sera from lambs that received colostral antibody (groups 1–4) and in controls (group 5)*

Group no.	Lamb no.	Day after inoculation											
		−1	+2	+4	+6	+8	+10	+14	+21	+42	+71	+139	+186
1	C/155	20	10	10	10	20	160	80	40	80	40	40	40
	156	20	20	10	20	40	160	320	80	40	40	40	20
	191	20	20	10	10	10	10	10	10	10	T	T	10
	192	20	10	10	10	20	40	80	20	20	10	10	10
	195	20	20	10	10	10	10	10	< 10	< 10	< 10	< 10	< 10
2	C/147	< 10	< 10	< 10	< 10	80	80	640	80	320	—	—	—
	149	< 10	< 10	< 10	10	80	80	160	320	320	—	—	—
	151	< 10	< 10	< 10	< 10	160	640	2560	2560	1280	—	—	—
	199	< 10	< 10	< 10	10	160	160	160	80	80	—	—	—
	201	< 10	< 10	< 10	< 10	160	320	1280	1280	5120	—	—	—
3	C/143	< 10	< 10	< 10	20	2560	2560	5120	640	640	—	—	—
	157	< 10	< 10	< 10	20	2560	2560	2560	320	320	—	—	—
	158	< 10	< 10	< 10	< 10	640	2560	D	D	D	—	—	—
	206	< 10	< 10	< 10	80	160	160	640	320	320	—	—	—
	210	< 10	< 10	< 10	80	1280	320	2560	640	640	—	—	—
4	C/185	< 10	< 10	< 10	40	2560	2560	2560	320	640	—	—	—
	190	< 10	< 10	< 10	320	2560	2560	5120	2560	2560	—	—	—
	193	< 10	< 10	< 10	80	2560	2560	2560	1280	1280	—	—	—
	213	< 10	< 10	< 10	10	2560	D	D	D	D	—	—	—
	214	< 10	< 10	< 10	< 10	320	640	1280	640	640	—	—	—
5	C/196	< 10	< 10	< 10	80	1280	1280	1280	320	320	320	160	320
	197	< 10	< 10	< 10	40	2560	1280	1280	1280	1280	1280	320	640
	198	< 10	< 10	< 10	160	1280	640	640	640	640	640	160	320
	226	< 10	< 10	< 10	160	2560	2560	10240	2560	1280	1280	640	640

T = incomplete inhibition detected at a dilution of 1/10.
D = dead.

of virus not exceeding 5 p.f.u. per 0·2 ml. were detected in the plasma of 2 of the 5 lambs in group 2 (Table 1). All the lambs in the other three groups developed viraemia between days 1 and 5 but only in one animal in group 3 did this persist until day 6, the titre being $10^{1.64}$ p.f.u. per 0·2 ml.

The titres of HI antibody in three of the lambs in group 1 increased and remained positive till at least 186 days after inoculation (Table 2). The antibody titre in one of the other animals declined and was negative by day 21 while the titre of the remaining animal declined to 1/10 which was maintained till day 42 after which serum from this animal caused either complete or partial inhibition of agglutination, only at a dilution of 1/10. The initial antibody responses of animals in group 2 were less than those recorded in the control lambs or lambs in groups 3 and 4; however, the titres recorded 21 and 42 days after inoculation were of the same order as in these groups. During the subsequent 144 days the titres recorded in the control group declined and ranged from 1/160 to 1/640 on day 186 after inoculation.

During the 9 days after rechallenge no virus was detected in the plasma of any lamb in Group 1. Four of the lambs showed an increase in HI antibody titre by day 4 which continued to rise and achieved maximum titres of between 1/320 and 1/1280 by day 9–14. The titres of none of these sera was affected by 2-ME. The remaining animal did not develop HI antibody until day 14 when a titre of 1/10 was recorded which was maintained until day 35 when the experiment was terminated. The titre of none of the samples from this animal was affected by 2-ME.

DISCUSSION

The titre of louping-ill HI antibody in the sera of lambs which receive colostral antibody from immune ewes has been found to be approximately the same as those of the dams (Williams & Thorburn, 1961; Brotherston *et al.* 1971). Titres in ewes that have recovered from natural infection are generally $\geqslant$ 1/160 (Williams & Thorburn, 1961; Smith *et al.* 1964) and similar titres were found after vaccination (Brotherston & Boyce, 1969, 1970). From the results presented here it is evident that experimental s.c. infection is completely inhibited in lambs with titres of maternal antibody $\geqslant$ 1/40; such titres will normally be achieved in lambs born to immune ewes. Lambs which receive such colostrum may therefore be assumed to be solidly protected, which is in accordance with the concept of Wilson & Gordon (1948), Williams & Thorburn (1961), Smith *et al.* (1964) and Smith (1969).

The half-life of colostrum-derived HI antibody was found to be 13·5 days (Brotherston, personal communication) which is in agreement with that of 13·7 days for IgG (Smith, Wells, Burrells & Dawson, 1976). HI antibody will decline from initial titres causing lambs to be refractory to infection to amounts where a reaction may occur. The five lambs infected when serum antibody was 1/20 did not become viraemic and four either developed a small rise in antibody titre or were sensitized, so that on later rechallenge an antibody response occurred rapidly and was entirely IgG, a feature characteristic of an anamnestic response. The immune response to rechallenge of the fifth animal although delayed and less, also produced only IgG, and therefore was also probably sensitized by the initial

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exposure to virus. Animals whose sera had become negative within 34 to 20 days, as measured by the HI test, either did not become viraemic or developed a viraemia of low intensity and a slightly delayed antibody response which however reached titres similar to control lambs after 21 days. Lambs that had been negative for longer periods, like the controls, developed viraemia and high titres of antibody. Lambs that receive colostral antibody to louping-ill virus are therefore protected for at least three weeks after maternal HI antibody can no longer be detected; under natural conditions this loss of antibody is unlikely to occur before lambs are about 2 months of age. In addition, such lambs will not develop a viraemia of sufficient intensity to infect the vector since this must be in excess of 10^3 p.f.u. per 0·2 ml. of blood (Beasley & Reid, in preparation).

In louping-ill endemic areas, lambs are generally born at or about the peak period of tick activity in the spring (MacLeod, 1940) and it has been found that during this season an adult sheep is parasitized by eight times as many ticks than is a lamb (Milne, 1947), which is therefore also the case for virus-infected ticks. Young lambs and adult sheep are equally susceptible to peripheral challenge with louping-ill virus (Reid & Doherty, 1971*a*; Doherty & Reid, 1971). Provided colostral antibody is effectively transferred, the proportion of lambs and ewes with antibody will be the same and greater losses may therefore be expected in adult animals.

Louping-ill virus infection in lambs is therefore concluded to be of minor importance as a cause of mortality and of little epidemiological significance.

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