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The epidemiology of louping ill in Ayrshire: the first year of studies in sheep

By C. E. GORDON SMITH, DOLORES A. McMAHON
London School of Hygiene and Tropical Medicine

K. J. O'REILLY
Wellcome Research Laboratories

A. L. WILSON and J. M. ROBERTSON
West of Scotland Agricultural College

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Following an outbreak of louping ill in lambs on a hill farm (Camlarg) near Dalmellington, Ayrshire, a study was made of the epidemiology of the disease on three neighbouring farms (Mossdale, Dalcairnie, Knockgray—shaded in Fig. 1). The affected area was that in which Gordon, Brownlee, Wilson & Macleod (1932) and Gordon (1934) did much of the original investigation into louping ill infection of sheep. These workers developed a formalinized sheep brain vaccine which has been used prophylactically in louping ill areas of the British Isles ever since. Generally, lambs are vaccinated once during the second 6 months of their life and this is the only artificial protection they receive. In the area studied, lambs are born on the hills in April and remain there until weaned in September. The male lambs are then sold off the farm and the female lambs (ewe hoggs) are sent to a tick-free lowland pasture (the wintering) from October until the following April. Generally speaking they return to the hirsel (an area of the hills confined naturally by hill burns, ravines, etc.) on which they were born and where they remain until they are sold as cast ewes at the age of $5\frac{1}{2}$–6 years. Black-face hoggs for Dalcairnie Farm are, however, born on Brownhill Farm some 7 miles away. Only cross-bred lambs are produced at Dalcairnie and all are sold in the autumn. The sheep on these farms are not mated (tupped) until November in their second year (as gimmers) after which they produce a lamb annually, usually for 4 subsequent years.

This paper reports the first year of work in a long-term study of which further reports will follow. The first year of work in sheep was mainly on Dalcairnie Farm which has two hirsels (Dalcairnie Hill and Barbeth) separated only by a stream and a farm road. Barbeth forms roughly the southern one-third of the farm.

MATERIALS AND METHODS

Virus. The Moredun strain of louping ill virus after 30–35 mouse passages was used.

Sera were stored at $-20°$ C.

Haemagglutinin. After exsanguination the brains of sick infected mice were removed and ground up in 0·4 % bovine albumin in borate-buffered saline of pH 9.

After centrifugation at 3000 r.p.m. for 10 min., the supernatant was spun at 13,000 r.p.m. for 1 hr. in a Spinco angle head. The supernatant was stored at 4° C. and used as antigen. Optimal haemagglutination occurred at pH 6·3.

Haemagglutinin-inhibition (HI) tests. The sera were twice extracted with acetone in the cold (Clarke & Casals, 1958), then absorbed with goose cells. Using M.R.C. pattern Perspex plates, 8–16 units of haemagglutinin in pH 9 borate saline were added to two-fold dilutions of extracted serum starting at 1/10 in the same buffer. After standing at room temperature for 1 hr., 0·25 % goose cells were added in a phosphate buffer which adjusted the reaction to pH 6·3. The cells were allowed to sediment at room temperature. Titres are stated as the reciprocal of the highest dilution causing complete inhibition.

Neutralization tests. These were carried out in 3- to 4-week-old mice. Inactivated sera were mixed with an equal volume of about 100 LD 50 of virus diluted in equal parts of fresh guinea-pig serum and 10 % horse serum broth. The mixtures were incubated for 1 hr. at room temperature then inoculated intracerebrally into groups of six mice. The virus was titrated at $\sqrt{10}$-fold dilutions in 50 % normal sheep serum. The results were computed using survival times by the method of Smith & Westgarth (1957). Sera showing a significant difference from the controls at the 5 % level were recorded as positive.

Virus isolations. Brains of lambs were placed in 50 % glycerol saline and sent to London. The brains were then ground up as a 20 % suspension in borate-buffered 0·75 % bovine albumin (pH 9) and inoculated intracerebrally into two litters of baby mice. The brains of sick or dead mice were tested, after passage if necessary for complement-fixing antigen against a standardized mouse louping ill antiserum.

RESULTS

Preliminary study on Camlarg Farm

Heavy lamb losses occurred on some farms in the area in the spring of 1960. In September of that year, forty ewe hoggs were bled on Camlarg Farm (Fig. 1) where about 50 % of the lambs had died from louping ill in the spring. HI antibody was found in twenty-four (60 %) and neutralizing antibody in twenty-five (63 %): as the animals were then 5 months old this is unlikely to have been maternal in origin. In March 1961, thirty-eight of them were bled again. Examination of the March sera showed that six (25 %) of the animals with HI antibody in the previous September had lost it less than one year after infection and the median titre of the positive sera had fallen from 160 to 20. The pattern of the change corresponds with an eight-fold drop in the titre of all the animals in 6 months. The prevalence of neutralizing antibody, on the other hand, was unchanged between September and March (Fig. 2).

Main study on Dalcairnie Farm

In 1961 all the ewes on Dalcairnie Farm were ear-tagged and bled on 29/30 March and 5 June. At the first bleeding, approximately two-thirds of this flock (207 ewes) were given formalinized sheep brain vaccine* and the remainder (99) were kept as

* Vaccine supplied by Burroughs Wellcome and Co.

controls. This was their second dose of vaccine, the first having been given 1–5 years previously as hoggs.

A sample of each group was bled 3 weeks after vaccination. Post-mortem examinations were made and virus isolation attempted from brain of some of the

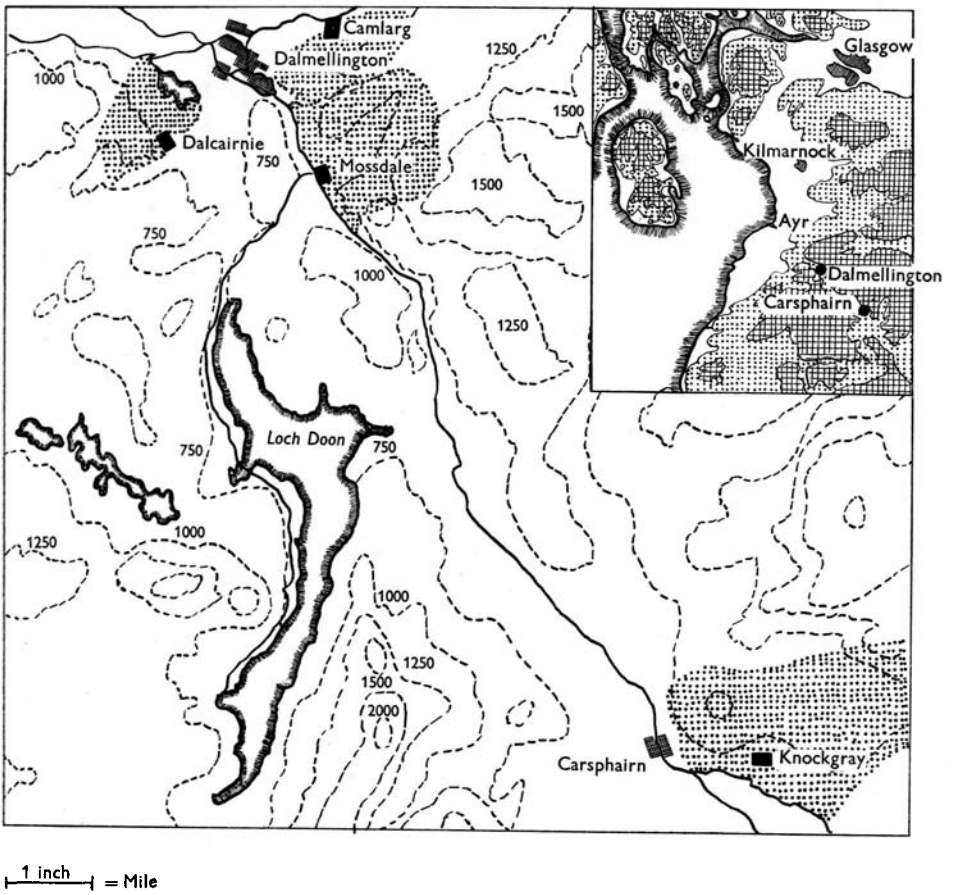

Fig. 1. Map showing the study areas in south-west Scotland.

lambs which died between March and June. Blood was also obtained from their dams as soon as possible after lamb death.

The distribution of ewes between the two hirsels of Dalcairnie Farm is shown in Table 1.

Haemagglutinin-inhibiting antibody in ewes

Of the Dalcairnie Hill ewes, 64 % had antibody in March compared with 35 % on Barbeth hirsel (Table 2). This difference is significant. On Barbeth hirsel the prevalence of antibody in both March and June was significantly higher in revaccinated (42 and 49 %) than control (20 and 18 %) animals. Of all the conversions (Table 3) from negative to positive antibody status, 67 % (10/15) at Dalcairnie Hill, and 20 % (1/5) at Barbeth, occurred in the 2-year-old ewes. Few conversions occurred at older ages. Two 2-year-old ewes from Dalcairnie Hill were

the only animals to show an increase in titre ⩾ 4-fold. Six ewes from the same hirsel (three of them 2 years old) and one from Barbeth lost antibody between March and June.

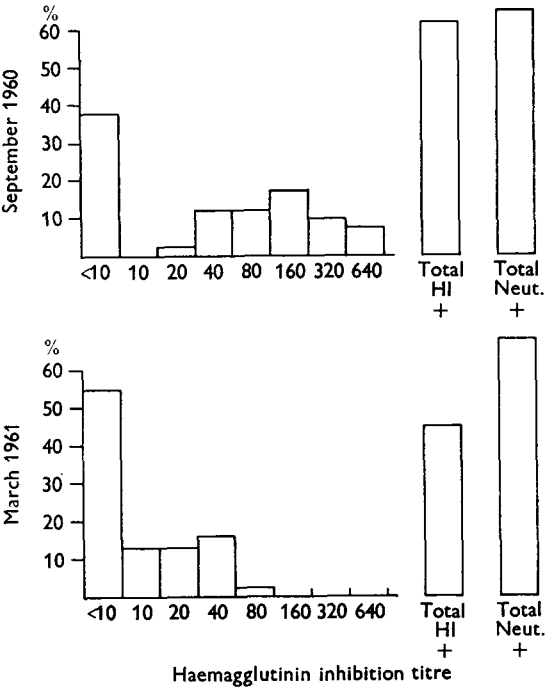

Fig. 2. The change in prevalence of antibody in the surviving lambs at Camlarg Farm where about 50 % died in May–June 1960.

Table 1. *The distribution of re-vaccinated and control ewes on the hirsels of Dalcairnie Farm*

	Dalcairnie Hill	Barbeth	Total
Revaccinated	143	64	207
Controls	69	30	99
Total	212	94	306

Neutralizing antibody in ewes

The preliminary study showed that HI antibody declines more rapidly than neutralizing antibody. The neutralization test was therefore mainly used to test sera without HI antibody. The distribution of the sera examined is shown in Table 4. The March and June sera from each animal were tested as far as possible in the same test.

No significant difference was found between the proportions with antibody in March (49 %) and June (67 %) on Barbeth (Table 5). However, there was a significant increase in prevalence between March (78 %) and June (97 %) on Dalcairnie Hill hirsel and there were significantly higher prevalences of antibody on Dalcairnie Hill than Barbeth in both March and June.

Table 2. *Haemagglutinin inhibiting-antibody in ewes on two neighbouring hirsels in* 1961

Hirsel	Age (years)	Control			Revaccinated			Total		
		March	June	% diff.	March	June	% diff.	March	June	% diff.
Dalcairnie Hill	2	7/15	11/15	+27	19/39	20/34	+10	26/54	31/49	+15
	3	17/21	16/21	−5	22/31	22/29	+5	39/52	38/50	+1
	4–6	26/33	24/30	+2	44/72	40/58	+8	70/105	64/88	+6
Total		50/69	51/66	.	85/142	82/121	.	135/211	133/187	.
%		73	77	+4	60	68	+8	64	71	+7
Barbeth	2	0/8	0/7	0	3/14	4/14	+7	3/22	4/21	+5
	3	2/9	1/9	−11	4/13	4/12	+2	6/22	5/21	−3
	4–6	4/13	4/12	+2	20/37	22/35	+9	24/50	26/47	+8
Total		6/30	5/28	.	27/64	30/61	.	33/94	35/89	.
%		20	18	−2	42	49	+7	35	39	+4

Table 3. *Haemagglutinin-inhibiting antibody conversions in paired sera from ewes on two neighbouring hirsels between March and June* 1961

Hirsel	Age (years)	No. tested	< 1 to ⩾ 1	⩾ Four-fold	Total	% conversion
Dalcarnie Hill	2	49	8	2	10	20
	3	50	2	0	2	4
	4–6	88	3	0	3	3
Total		187	13	2	15	8
Barbeth	2	21	1	0	1	5
	3	21	0	0	0	0
	4–6	47	4	0	4	8
Total		89	5	0	5	6

An estimate of the total cumulative infection rates can be obtained by adding to the number of sheep with HI antibody, a proportion derived from the sample tested for neutralizing antibody. This is calculated separately for each age group, place and for March and June.* The percentage figures which result give estimates of the proportion of the flock which has been naturally infected at some time during their lives (Table 6).

Table 4. *Sera from ewes examined for neutralizing antibody*

	Dalcairnie Hill	Barbeth	Totals
Revaccinated	42 (10)	31 (1)	73 (11)
Controls	45 (31)	14 (2)	59 (33)
Totals	87 (41)	45 (3)	132 (44)

Figures in parentheses = number with HI antibody.

Measure of agreement between HI and neutralization tests

The antibody status of 132 ewes bled in March and June and tested in both tests is shown in Table 7: 103 sera were positive and 58 negative in both tests. Thus, the two tests agreed in 61 % (161/262) of the sera examined. Neutralizing but not HI antibody was found in 39 % (101/262). Only two sera with HI antibody gave negative neutralization tests.

Antibody conversions between March and June

There was no change in 90 % of the sheep with both types of antibody (Table 8) in 73 % of those with neutralizing but not HI antibody, or in 35 % of those with neither antibody. 30 % of those without either type of antibody in March had both types by June, while 35 % developed neutralizing antibody only; thus altogether 65 % of these antibody-free sheep acquired neutralizing antibody by June. Of those with only neutralizing antibody in March, 19 % developed HI antibody by the summer.

In sera examined by both tests (Table 5) HI and neutralizing antibody responses followed a similar pattern. On Dalcairnie Hill 88 % (15/17) of the animals showing a neutralizing antibody response and 71 % (12/17) of those with a HI response were 2-year-old ewes. Six of the animals which had neutralizing antibody responses did not develop HI antibody. Nine animals showed both HI and neutralizing antibody responses. Another three animals with neutralizing antibody in March and June developed HI antibody. Three of the four Barbeth 2-year-old ewes which showed neutralizing antibody responses failed to show HI antibody conversions. In the older age groups on both hirsels, there were six

* E.g. Dalcairnie Hill, 3–6 years, June. Table 2: 36 of 138 had no HI antibody. Table 5: 16 of 17 without HI antibody had neutralizing antibody, i.e. 0·941. Therefore add $0{\cdot}941 \times 36$ to the number with HI antibody in Table 2, i.e.

$$\frac{0{\cdot}941 \times 36 + 102}{138} = \frac{34 + 102}{138} = \frac{136}{138} = 99\,\%.$$

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Table 5. *Neutralizing and haemagglutinin-inhibiting antibody and conversions in ewes tested by both tests on two neighbouring hirsels in* 1961

			Neutralizing antibody				HI antibody			
Hirsel	Age (years)	No. pairs of sera examined	March	June	Conversions of negative sera	(%)	March	June	Conversions of negative sera	(%)
Dalcairnie Hill	2	41	24	39	15	88	16	26*	12	48
	3	23	23	23	0	0	14	15†	2	22
	4–6	23	21	22‡	2	(100)	11	14	3	25
Total		87	68	84	17	.	41	55	17	.
%		.	78	97	89	.	47	63	37	.
Barbeth	2	16	3	7	4	31	0	1	1	6
	3	12	6	8‡	3	(50)	2	2	0	0
	4–6	17	13	15‡	3	(75)	1	3†	3	19
Total		45	22	30	10	.	3	6	4	.
%		.	49	67	43	.	5	12	10	.

* Two ewes lost haemagglutinin-inhibiting antibody between March and June.
† One ewe lost haemagglutinin-inhibiting antibody between March and June.
‡ One ewe lost neutralizing antibody between March and June.

Table 6. *Estimated cumulative percentage infection rates in ewes*

Age (years)	Dalcairnie Hill			Barbeth		
	March	June	Change	March	June	Change
2	67	98	+31	27	52	+25
3–6	98	99	+ 1	80	89	+ 9

animals with neutralizing antibody in March which developed HI antibody in June and four HI antibody-free animals which acquired neutralizing antibody only. One ewe from Dalcairnie Hill and two from Barbeth lost neutralizing antibody and four from both hirsels lost HI antibody (Table 5). There was a significantly higher proportion of HI and neutralizing antibody conversions on Dalcairnie Hill than on Barbeth.

Table 7. *Comparison of the prevalence of HI and neutralizing antibody in ewes*

		Neutralizing antibody				
		March		June		Total
		+	−	+	−	
HI antibody	+	42	2	61	0	105
	−	48	40	53	18	159
Total		90	42	114	18	264

Table 8. *Summary of antibody conversions*

		June							
			Gain						
March		No change	HI N	%	HI only	%	N only	%
HI+ N+	42	38	90	.	.	.	.	.	.
HI+ N−	2	0	0	.	.	.	.	2	100
HI− N+	48	35	73	.	.	9	19	.	.
HI− N−	40	14	35	12	30	.	.	14	35

Table 9. *The distributions of HI antibody titres before and after revaccination*

	Before revaccination			After revaccination	
	< 10	⩽ 10	Median	Four-fold increases	Median
Revaccinated	10	18	20	3	20
Control	5	12	40	1	40

Effects on revaccination

Seventeen control ewes and twenty-eight revaccinated ewes were bled 3 weeks after the vaccination of two-thirds of the flock. The distributions of HI antibody titres before and after revaccination are shown in Table 9. Clearly there was no difference between the two groups on this basis. The four-fold increases in antibody in both groups may have been due to natural infection.

Twelve of these pairs of sera (4 controls and 8 vaccinated) were tested for neutralizing antibody. Ten had neutralizing antibody in both specimens, two became positive and one lost antibody. Again, no difference between the groups was demonstrated, although the number tested was rather small.

Antibody in lambs

Lamb sera were obtained at the June bleeding and compared by the HI and neutralization tests with the corresponding maternal sera. HI antibody was present in sera from 16 % (12/76) of lambs from the control ewes and from 22 % (29/131) of lambs from the vaccinated ewes. There is no significant difference. There is, however, a barely significant difference on Barbeth between the lambs of control ewes (0/25) and of vaccinated ewes (6/43:14 %).

Table 10. *Haemagglutinin-inhibiting antibody in simultaneous samples from mothers and lambs in June* 1961 *at Dalcairnie Farm*

Mother's antibody	Antibody in lambs					
	< 1	1	2	3	4	5
< 1	**80**	**5**	**1**	.	.	.
1	**31**	**4**	**2**	.	.	.
2	40	**8**	**5**	**1**	.	**1**
3	18	5	**2**	.	.	.
4	2	.	**1**	.	**1**	.
5	1	.	1	1	.	.

Antibody titres: 1 = 1/10, 2 = 1/20, 3 = 1/40 etc.

Table 11. *Relationship of ewe and lamb haemagglutinin-inhibiting (HI) and neutralizing (N) antibody in June*

Ewe	Lamb			
	HI+ N+	HI− N+	HI− N−	Totals
HI+ N+	0	6	1	7
HI+ N−	0	0	1	1
HI− N+	4	10	5	19
HI− N−	0	0	6	6
Totals	4	16	13	33

When the titres of HI antibody in lambs were compared with those of their mothers, 7 % (6/86) of lambs from mothers without detectable antibody in June had antibody. Apart from these, only one lamb had a titre four-fold greater than its mother. On the other hand, sixty-nine had titres at least four-fold less than their mothers. Two-thirds (139/210) of lambs had similar amounts of antibody to their mother (Table 10). In only one case in ten pairs of lambs suckled by the same mother was there any disagreement between the HI antibody status of the twins: one was positive and the other negative.

Of these lamb sera, thirty-three were examined by the neutralization test and twenty-one had neutralizing antibody. In twenty instances where the lamb had neutralizing antibody, the June sample from the ewe was also found to have neutralizing antibody. Only four of the lamb sera had both types of antibody. The remainder had either the same neutralizing antibody status (48 %) or less than their mothers (39 %) (Table 11).

Lamb deaths

Three of the thirteen freshly dead lambs examined post mortem died from pyaemia. Brains from eight were inoculated intracerebrally into mice and louping ill virus was isolated from one Dalcairnie Hill lamb. Another Dalcairnie Hill lamb had louping ill but recovered. Tick pyaemia killed both the Barbeth lambs. Six of the dead lambs were born to gimmers (Table 12).

Sentinel hoggs

As part of a vaccination experiment to be reported later, twenty-two hoggs from tick-free land were released on Dalcairnie Farm on 25 April: ten on Barbeth and twelve on Dalcairnie Hill hirsels. On Barbeth, within 6 weeks, there was one fatal clinical case (unconfirmed in the laboratory) of louping ill and one was destroyed after falling over a cliff: a probable infection rate of 11 % (1/9). On Dalcairnie Hill, within the same period, there were two deaths from pneumonia, four confirmed louping ill deaths and one (or possibly two) with serological (HI and neutralizing antibody) evidence of louping ill infection: a probable infection rate of 50–60 % (5–6/9).

DISCUSSION

This paper sets the scene of a long-term study to elucidate the epidemiology of louping ill infection and hence to design improved control measures. Louping ill, like the other arthropod-borne encephalitides, is essentially a biphasic illness. Following tick-bite and virus multiplication in the cells initially infected, viraemia occurs and infection becomes widespread in the animal. This visceral phase causes only relatively mild symptoms, except possibly in young lambs. The visceral phase cannot, of course, be clinically diagnosed as louping ill. The virus may or may not succeed in reaching the cells of the central nervous system: if it does then the second phase of encephalitis follows: namely clinically recognizable louping ill. It is important to be clear about this because in preventing clinical and fatal louping ill we are essentially concerned in preventing the encephalitic phase (i.e. preventing spread of the virus to the central nervous system).

Infection of the central nervous system depends on the inability of the defence mechanisms to prevent the virus penetrating the blood-brain barrier. With the related West Nile virus, primary virus multiplication appears to occur in the walls of the blood vessels (Kundin, Liu, Hysell & Hamachige, 1963). The encephalitic phase therefore depends on the degree and duration of viraemia which is largely determined by the rate of development of circulating antibody. Factors which impair the defence mechanisms will increase the probability of encephalitis, for example:

(1) *Age.* Newborn animals (e.g. baby mice) are generally much more susceptible to arbovirus infections than older animals. Either higher virus concentrations are reached because of greater cell susceptibility or the defence mechanisms are incompletely developed.

(2) *Nutritional state.* Edward (1947) reported difficulty in producing encephalitis in sheep following the inoculation of virus subcutaneously during the summer

Table 12. *Disease in lambs at Dalcairnie Farm in* 1961

	Lamb			Mother				
					HI antibody			
Date of death	Post-mortem findings	Isolation of LI virus from brain	Age	Hirsel	Pre-vaccination	Post-vaccination	At lamb death	5. vi. 61
13. v.	NAD*	–	2	DH	< 1	.	1	< 1
13. v.	NAD	+	2	DH	< 1	< 1	< 1	< 1
23. v.	NAD	–	2	DH	< 1	.	< 1	1
24. v.	Pyaemia	–	2	DH	2	3	.	2
27. v.	NAD	ND†	2	DH	⩾ 6	.	.	3
31. v.	NAD	ND	2	DH	< 1	.	.	< 1
27. iv.	NAD	–	3	DH	< 1	.	< 1	1
10. v.	NAD	–	3	DH	< 1	.	< 1	< 1
13. v.	ND	–	3	DH	3	3	< 1	.
24. v.	Pyaemia	–	4	B	2	.	.	2
28. iv.	Pyaemia	ND	4	B	2	.	.	1
Survived	Clinical	HI: 3 on 5. vi. 61	4	DH	< 1	.	< 1 (24. v. 61)	. .
14. v.	ND	ND	?	DH	< 1	.	1	.
14. v.	ND	ND	?	DH	< 1	.	1	< 1

* NAD = no abnormality detected. † ND = not done.

HI antibody titres. 1 = 1/10, 2 = 1/20, 3 = 1/40, etc.

months when their general health and nutrition was good. In the vaccination experiment mentioned above (p. 62), fourteen susceptible hoggs were exposed on 25 March on Knockgray Farm at Carsphairn about 10 miles south of Dalmellington. At the end of June none showed any serological evidence of louping ill infection. They were then exposed on Camlarg Farm near Dalmellington and although none showed evidence of clinical disease, thirteen (93 %) had developed louping ill neutralizing antibody and nine HI antibody by the following September. In the group of similar hoggs exposed on Camlarg Farm from March to June, 100 % became infected and 50 % died of louping ill. Whether this difference between exposure in March and exposure in July was due to age or nutritional factors or to some other cause is unknown.

(3) *Concurrent disease.* Gordon *et al.* (1932) and MacLeod (1962) found that the simultaneous infection of sheep with tick-borne fever and louping ill significantly increased the incidence of encephalitis and the frequency of isolations of virus from their brains. Other diseases which occur during the same period, tick pyaemia, *Pasteurella* pneumonia, anaerobic infections, may also increase the risk of encephalitis.

(4) *Cold and increased blood carbon dioxide.* Sellars & Lavender (1962) showed in mice that increased carbon dioxide tension in the blood increased the permeability of the blood-brain barrier to virus and thus susceptibility to encephalitis. They also found that exposure to cold (4° C. for several days) increased the CO_2 tension in the blood. Muscular exercise is also known to increase the risk and degree of paralysis in poliomyelitis. Obviously both cold and exercise, and the concurrence of any disease causing an increase in blood CO_2 may be contributory factors in louping ill encephalitis.

Vaccination

In weaned animals there is evidence from laboratory studies (Edward, 1947), from field trials (Gordon, Brownlee, Wilson, & Macleod 1962) and from the consensus of opinion over the last 30 years, that vaccination with formalinized sheep brain vaccine prevents a high proportion of animals infected from developing encephalitis. Vaccination does not cause detectable antibody formation and as we have shown here, a second dose 1–4 years after the initial dose received as a hogg also does not give an antibody response. Wilson & Gordon (1948) inoculated sheep with 3–4 doses at 2-week intervals before detectable neutralizing antibody appeared. Young animals are vaccinated during their first winter and this appears to sensitize them so that when they are naturally infected their defence mechanisms are accelerated and the illness is limited to the visceral phase. Vaccination therefore does not prevent infection but does prevent encephalitis.

In this south-west part of Scotland, louping ill mainly affects young lambs, often in sharp epizootics such as that at Camlarg Farm described above. The problem, therefore, is in the unweaned lambs of vaccinated ewes.

The protection of young lambs does not depend directly on vaccination but on maternal antibody. A vaccinated ewe will not confer sensitization on her lamb so that unless she has had a natural infection, her lamb will be susceptible to infection

and probably death. Maternal antibody resulting from a natural infection should, however, protect a lamb throughout its first season of maximum tick biting activity.

In the preliminary investigation, an eight-fold drop in HI antibody was observed between 6 and 12 months after infection, 25 % of the animals thus becoming HI negative. In another experiment 33 % of vaccinated and experimentally infected hoggs lost HI antibody within 6 months of infection (O'Reilly, Smith & White, unpublished). Neither in the field investigation nor the laboratory experiment was there any reduction in the proportion with neutralizing antibody during the period of observation. Therefore, HI antibody is probably evidence of relatively recent infection, while neutralizing antibody indicates natural infection at any previous time. Maternal antibody of both types is transmitted to lambs.

Tick drags were made over the farms at intervals throughout the year and the main activity of nymphal *Ixodes ricinus* was found in April/May and the larval peak in May. Later studies suggest that the nymphal peak is a little earlier than the adult peak (Varma, *et al.* unpublished). Most, if not all of the infections of lambs and sheep must be attributed to the bites of adults or nymphs. Nymphal activity is maximal during or shortly after lambing and the peak of adult activity probably after the completion of lambing. The period of tick-biting activity varies from year to year according to the weather conditions. If infections occur 2–3 weeks before lambing then it is possible that previously non-infected ewes will be able to provide ample maternal immunity. In all probability (and certainly in 1962 and 1963) most of the tick activity is too late and protection of the lambs is dependent on infection in the ewes or hoggs in previous years.

The course of events in the latter situation is shown in Fig. 3; if the infection rate is too low for every hogg to be infected, some gimmers in the following year will have susceptible lambs, and with still lower infection rates, also older ewes. Thus with persistent annual infection rates of 40–60 %, the expectation of louping ill in lambs of gimmers would be about 25 % and in the whole flock about 12 %. With persistent infection rates over 90 % less than 2 % of all lambs would be expected to die of louping ill. This latter state is probably reached on some farms where louping ill is hyperendemic. When the tick-biting is early enough to immunize the ewes in the spring before lambing, the proportion of gimmers' lambs affected would be halved for infection rates of 40–60 % and reduced to nil for a 90 % rate.

Protective measures can be intelligently designed only with a knowledge of the prevailing infection rate and of changes which may occur in it. Where the infection rate is consistently very high (about 90 %) no clinical louping ill or almost none will be seen. Constant infection rates between 20 and 60 % can be calculated to give about the same overall lamb losses (11–13 %) and there are probably many farms with this level of morbidity. However, in those areas where louping ill occurs in periodic epizootics we have shown that the infection rate does not remain constant but fluctuates. The infection rate in sheep appears to fall after periods of high rate: later work on Dalcairnie Hill has shown that in 1962 there was an infection rate of about 35 % as compared with about 60 % in 1961.

There is little doubt that the present methods of vaccination are highly effective

in the presence of consistent high infection rates. With lower and fluctuating rates, vaccinated animals are well protected but a proportion of young lambs are not protected by maternal antibody.

Clearly in such situations any improved vaccine must be primarily designed to prevent disease in young lambs. Either it must itself produce such a level of antibody in the mother that all her subsequent lambs will be passively immune, or it must not prevent natural infection while protecting her from encephalitis. In the latter case it must be remembered that the bite of a tick probably inoculates virus directly into the bloodstream where it is immediately exposed to circulating antibody, and therefore, any such new vaccine must be tested by feeding infected ticks on vaccinated animals to determine whether natural infection can regularly occur causing a good antibody response.

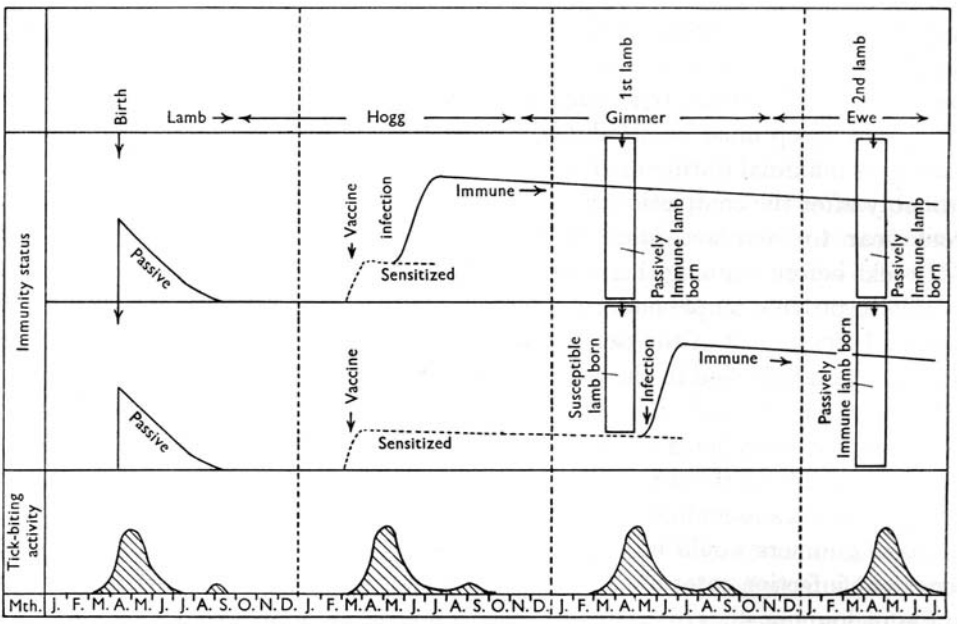

Fig. 3. Diagrammatic representation of the probable mechanism of vaccination in the control of louping ill.

Differences in infection rate between hirsels

Infection rates vary markedly between farms and between hirsels on an individual farm. The infection rate on Barbeth hirsel was significantly lower than on Dalcairnie Hill hirsel. The various estimates of the infection rates are shown in Table 13.

Barbeth is a sloping well-drained hirsel while Dalcairnie Hill has large areas of swampy ground with thick grass and rushes. Although comparative studies have not yet been made, the tick population on Dalcairnie Hill is probably higher than on Barbeth causing the difference in infection rate. It is notable that of the four farms on which we have studied tick populations, infection is absent only on Knockgray where the tick population seems to be lowest.

On the approximate basis of the means of these infection rates and the assumption that all lambs of mothers with antibody were protected, the expected numbers of lamb deaths from louping ill (2–3 on Barbeth and 1–9 on Dalcairnie Hill) were only a little higher than the observed numbers (0 on Barbeth and 1 on Dalcairnie Hill). The latter may well be underestimated because of failure to isolate virus from animals dead for some time before autopsy. Clearly even in the presence of a high louping ill infection rate there may be little or no clinical louping ill if the sheep population has been heavily infected in preceding years.

Table 13. *Estimates of the infection rates on the two hirsels on Dalcairnie Farm*

	Gimmers		
	HI conversions	Neut. conversions	Sentinel hoggs
Hirsel	(%)	(%)	(%)
Dalcairnie Hill	48	88	50–60
Barbeth	6	31	11

Twin lambs

Where there are twin lambs, one lamb sometimes acquires a lower level of passive immunity than the other. Batty, Thomson & Hepple (1954) have found a similar situation in anaerobic infections and Prydie (personal communication) with distemper antibodies in puppies. In this survey there was one of ten pairs of lambs in which at an age of 6 weeks one lamb had HI antibody and the other had not. In another experiment one of three pairs of lambs showed a similar discrepancy in neutralizing antibody at the same age, neither having HI antibody (O'Reilly, Smith and White, unpublished).

SUMMARY

1. Following an epizootic of louping ill on certain farms in south-west Ayrshire in 1960, a long-term study of several farms was initiated.

2. The flocks on two hirsels of one farm were studied during spring and early summer of 1961. Although only one lamb death was confirmed as due to louping ill, the infection rates in sentinel hoggs on the two hirsels were 50–60 % and 11 % respectively. The difference between the hirsels is probably attributable to the difference in the amount of tick habitat on them.

3. The ewes were bled in March and June and their lambs in June. Haemagglutinin inhibition (HI) and neutralization tests revealed that the HI antibody is much shorter lasting than neutralizing antibody. Many ewes, therefore, had neutralizing but not HI antibody. Otherwise agreement between the tests was good. In March almost all the ewes aged 3 years or more had antibody. Of the gimmers (2-year-olds) about two-thirds on one hirsel and one-third on the other had antibody in March: by June almost all the former and about half of the latter had antibody.

4. About two-thirds of the lambs had the same antibody status as their mothers in June and almost all the rest had less antibody than their mothers. Serological evidence suggestive of louping ill without recognizable clinical disease was found in six lambs and a further lamb recovered from clinical disease.

5. Revaccination of two-thirds of the flock failed to cause any detectable change in antibody status.

6. The epidemiology and pathogenesis are discussed in relation to immunity and infection rates, and to the design of control measures.

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