J. Hyg., Camb. (1971), **69**, 479
Printed in Great Britain

Field trials of an inactivated oil-adjuvant vaccine against louping-ill (Arbovirus group B)

By J. G. BROTHERSTON
Moredun Institute, Edinburgh
C. C. BANNATYNE, A. O. MATHIESON and T. B. NICOLSON
Scottish Veterinary Investigation Service

(*Received* 22 *March* 1971)

SUMMARY

A single dose of inactivated louping-ill oil-adjuvant vaccine elicited a serologically detectable immune response in sheep lasting for at least 1 year. These sheep when exposed to a natural focus of louping-ill virus were completely protected from clinical disease and 1 year after vaccination were able to pass on a substantial maternal immunity to their lambs.

Twenty-nine per cent of unvaccinated sheep, exposed at the same time, died from clinical louping-ill; half of the survivors showed positive sero-conversion and became immune, while the other half remained susceptible. The incidence of fatal encephalomyelitis in sheep which were known to have circulated virus exceeded 50 % in 2 out of 3 trials conducted simultaneously in different locations in Scotland in 1969.

INTRODUCTION

Severe clinical louping-ill mainly affects 1-year-old sheep when they are returned to tick-infested pastures in the spring after wintering on tick-free ground. By this time any passive immunity which they may have acquired from their dams has waned. Young lambs born on hill areas are also at great risk, if their dams are not actively immune, because the initial protection of lambs depends entirely upon passive maternal immunity acquired from the colostrum within the first 36 hr. of birth (Brambell, 1958).

Until 1967 a formalin-inactivated louping-ill vaccine was available to protect sheep, but because of danger to laboratory staff engaged in production the manufacture was discontinued. Until an acceptable alternative was found, the main method of combating tick-borne encephalomyelitis (TBE) was no longer available to those engaged in hill farming in areas where natural foci of louping-ill virus existed in Britain.

Although in the past the vaccine had apparently successfully reduced the incidence of clinical louping-ill in hill sheep (Gordon, Brownlee, Wilson & Macleod, 1962) its mode of action presented some unique features. A single dose did not induce a serological response and did not protect the majority of animals from the circulation of virus following challenge (Williams & Thorburn, 1961). However,

when studying the persistence of antibodies to louping-ill, O'Reilly *et al.* (1968) observed that although ewes did not respond serologically within 21 days of vaccination, when subsequently challenged with virus, they produced haemagglutinin-inhibiting (HI) and neutralizing antibodies in their sera and maternal immunity was transferred to their lambs. During studies on the epidemiology of louping-ill the view was expressed by Smith *et al.* (1964) that the use of a vaccine which prevented systemic infection with louping-ill virus in ewes but did not of itself induce maternal antibody might aggravate the disease incidence in young lambs exposed to consistently high foci of infection. Smith (1969) suggested that the vaccine may have been effective because it was poorly immunogenic; it gave a measure of protection from encephalomyelitis but did not prevent a systemic infection with virus which, if experienced within the first 2 years after a sensitizing dose of vaccine, enabled ewes to acquire active immunity and thereafter transmit maternal immunity. Clearly, under these circumstances the old method of protection which had apparently acted successfully for 30 years would indeed require replacement by a very good vaccine.

A new process of preparing an inactivated antigen from tissue cultures infected with the virus of louping-ill and investigations of the immunogenic properties have recently been described by Brotherston & Boyce (1969, 1970). The antigen, when emulsified in mineral oil, constituted a potent vaccine, and one dose gave a response measurable by HI and neutralizing antibodies within 10 days of subcutaneous injection. This response was accompanied by a lasting protection against challenge with virulent louping-ill virus and sheep which had never been exposed to infection passed maternal antibodies to their lambs. This paper describes trials to study the ability of the vaccine to protect sheep introduced to tick-infected pastures and the lambs born subsequently to some of them.

MATERIALS AND METHODS

It was decided to introduce small groups of unacclimatized sheep, some of which were vaccinated with oil-adjuvant vaccine, to farms where louping-ill was reported to be endemic, rather than to use indigenous stock. Unacclimatized sheep were defined as those under 1 year old, moved to hill pastures on which they had no previous experience of the grazing limits and where they would experience all the hazards of a new environment for the first time, including tick infestation. In this way the history, progress and serological status of the test animals was well documented, adequate controls were established and there was minimum disturbance to the sheep stocks on the farms selected for the experiments. Under these circumstances the test of the capability of the vaccine was severe, but was based upon calculations obtained from trials conducted previously at Moredun Institute.

Sheep

Eighty-seven Blackfaced ewe hoggs 10 months old were purchased in March 1969 from a farm on the lower slopes of the Pentland Hills, which are tick-free; serological tests indicated that they had no previous antigenic experience of loup-

ing-ill virus. They were individually numbered with ear tags and divided into 3 groups of 28 animals, of which 16 were given louping-ill vaccine and 12 were left unvaccinated. Three vaccinated animals were retained at Moredun Institute to monitor their immunity on tick-free ground during the ensuing months. The sheep were kept at pasture until 16 April 1969, when they were distributed to a farm in Selkirkshire, one in Perthshire and one in Argyllshire, selected as likely to be infested with ticks carrying louping-ill virus. Fourteen days before this they were dipped, dosed with an anthelmentic and given a booster dose of anti-clostridial vaccine. On the farms they were introduced to hill pastures at the same time as the ewe hoggs returned from wintering. Ten Blackface rams on the Argyllshire farm considered to have acquired natural immunity were made available to observe the effect of vaccination on indigenous stock.

Louping-ill vaccine, serological tests, virus isolation and criteria used to evaluate immunity

Louping-ill oil-adjuvant vaccine, type E, batch no. SK4, was injected subcutaneously just above the point of the brisket in a quantity of 0·5 ml. A local reaction developed in about 10 % of the animals but subsided in 14 days to a stage where it was difficult to detect by palpation. The vaccine had previously been tested in sheep and elicited an HI antibody response at a serum dilution of 1/160 and a neutralization index of $\log_{10}$ 2·6, 10 days after injection, when given in a dose of 0·25 ml. (half of that used in the trial). Haemagglutinin-inhibition (HI), neutralization tests and the preparation of vaccine were conducted as described by Brotherston & Boyce (1970). Sheep with an HI antibody titre of 1/10 or greater were shown to have significant virus-neutralizing properties in the serum and positive tests are detectable for at least 2 years after successful vaccination.

The technique of virus isolation from suspected tissues has been described by Doherty (1969) and the identification of virus by plaquing techniques by Reid & Doherty (1971). The neuropathological criteria of louping-ill encephalomyelitis were those of Doherty & Reid (1971). The protection afforded by the vaccine and the incidence of natural infection were estimated from the antibody (HI) response of the vaccinated and unvaccinated animals and by recovery of virus or the presence of neuropathological evidence of infection in animals which became ill or died between March and October 1969.

Supervision of the sheep

Arrangements were made with the shepherds to look over the sheep regularly on their rounds and to report unusual events to the Veterinary Investigation Laboratory. In addition the farms were visited each month and all of the animals were blood-sampled three times between May and October, when the survivors were returned to Moredun Institute. At the time of the first blood test smears were also taken to identify those which had become infected with tick-born fever (Foggie, 1951).

When an animal was found sick it was removed to the Veterinary Investigation Laboratory to establish a diagnosis and in addition serum and brain samples were dispatched to Moredun Institute to test for evidence of infection with louping-ill

virus. Dead animals were similarly treated with all dispatch. The Veterinary Investigation Officers were not aware of the vaccine category of the individual animals until after all the diagnostic criteria had been completed and the various reports collated.

Vaccination of animals recovered from natural infection

The identity was noted of a group of ten rams exposed to tick-infested pastures in 1968 on the farm in Argyllshire and likely to have recovered from infection. They were bled to establish their serological status in May 1969, and some of them were vaccinated.

Maternal immunity from vaccinated animals

Twenty of the animals on the farm in Selkirkshire in October 1969 were left to run with the homebred stock of in-lamb ewes. Those unvaccinated at the beginning of the trial, whether they showed serological evidence of recovery from natural infection with louping-ill or not, were vaccinated with a dose of the same vaccine, batch SK 4, which had been stored at 4° C. They were tested in May 1970 and the lambs were examined for evidence of maternal antibodies in their sera within 14 days of birth.

RESULTS

All the vaccinated animals had an adequate immunity, as judged by the HI test, before being transferred to the selected hill farms, and the titres of individual animals ranged from 1/10 to 1/640, with a mean of over 1/100. Three weeks after arrival at the farms most of the animals had settled to a regular grazing routine and it was established that the sheep in Selkirkshire and Argyllshire had become infected with tick-borne fever.

Selkirkshire farm

Three or four sheep consistently wandered apart from the flock because the spring of 1969 was generally late and the grazing in Selkirkshire was particularly poor, snow being on the hills until the end of April. The occurrence of clinical disease is shown in Table 1.

Three sheep were observed to become ill and two of them, both vaccinated (3K 58 and 3K 81), died or were killed for examination within the first 16 days. Both had severe pneumonia and septicaemia, and *Pasteurella haemolytica* type T was recovered from the lungs, livers and spleens. The third animal (4K 25) was unvaccinated and found dead on the 15th day, but no definite diagnosis could be established. In all three there was no evidence of infection with louping-ill. One other vaccinated animal (4K 87) died on the 22nd day, and although no cause could be defined, once again no evidence of louping-ill was detected by virus isolation procedures or neuropathology.

The first death attributable to louping-ill in unvaccinated sheep occurred in 4K 17 on the 18th day. Three others died on the 19th, 22nd and 39th day and in every case virus was isolated from the brains and neuropathological lesions were

present. At the end of the trial period 4 of the 12 unvaccinated sheep had died with louping-ill and 1 from unknown causes, 3 showed serological conversion and had acquired natural immunity, while 4 remained susceptible. Thirteen of the original 16 vaccinated animals remained healthy.

Table 1. *Occurrence of clinical disease after introduction of sheep in Selkirkshire*

Sheep no.	Treatment	Days after introduction	Diagnosis: Neuropath.	Diagnosis: Virus isolation	Cause of death
3K58	V	14	–	–	*Pasteurella* septicaemia
4K25	C	15	–	–	Not established
3K81	V	16	–	–	*Pasteurella* septicaemia
4K17	C	18	+	+	Louping-ill
4K24	C	19	+	+	Louping-ill
4K87	V	22	–	–	Not established
4K18	C	22	+	+	Louping-ill
4K11	C	39	+	+	Louping-ill

V = vaccinated.
C = unvaccinated control.
– = no lesions or virus isolation.

Perthshire farm

All 28 animals remained healthy and serum samples and smears showed no evidence of virus circulation or infection with tick-borne fever. They were all withdrawn in October, 6½ months after their introduction, except one vaccinated animal which had lost its identity tag and was recovered later. The HI tests of the vaccinated sheep, however, served as very useful controls in comparison with the serological status of vaccinated animals on the other farms, some of which had been exposed to challenge with virus.

Argyllshire farm

After the introduction of the animals the sequence of events was as follows. In contrast to central and southern Scotland, it was a mild spring in the west and the sheep quickly acclimatized. They were examined early in May and were found to be heavily infested with ticks. Blood smears indicated that the animals had been infected with tick-borne fever, but at this time there was no serological evidence in the unvaccinated animals of infection with louping-ill.

The first case of louping-ill was diagnosed in an unvaccinated sheep, which died on 23 May, 40 days after introduction. Five months later, in September, a second control animal died from louping-ill after 160 days at pasture and two others showed serological evidence of infection. The animals were returned in October and on arrival at the Institute a fifth unvaccinated animal was found to be ill. After 5 days with severe posterior paralysis it was killed and neuropathological examination of the brain and spinal cord showed extensive non-suppurative encephalomyelitis characteristic of louping-ill. The HI titre was 1/1280, and blood

values for calcium and magnesium were within the normal range, but virus was not isolated from the brain. A sixth animal, also unvaccinated, had an HI titre of 1/640 but appeared normal. Virus circulation had occurred in 6 of 12 unvaccinated sheep in this group, 3 of which died of louping-ill, 3 became naturally immune and 6 remained susceptible. The 16 vaccinated animals remained healthy throughout.

Combined results

The final assessment of the consolidated results of the trials on all three farms are shown in Tables 2 and 3. Of the 48 vaccinated sheep, three died from causes other than louping-ill and all of these were from the same farm in Selkirkshire

Table 2. *Unacclimatized BF ewe hoggs turned out between April and October in three hill areas in Scotland*

Area	Vaccinated		Unvaccinated	
	Died louping-ill	Total died	Died louping-ill	Total died
Selkirkshire	0/16	3/16*	4/12	5/12†
Perthshire	0/16	0/16	0/12	0/12
Argyllshire	0/16	0/16	3/12	3/12
	0/48	3/48*	7/36	8/36†

* Two died of Pasteurellois, one of unknown causes.
† One died of unknown causes.

Table 3. *Unvaccinated sheep exposed to foci of louping-ill virus*

	No. exposed	No. circulating virus	No. died of louping-ill	No. immune	No. susceptible
Argyllshire	12	6	3	3	6
Selkirkshire	12*	7	4	3	4
	24*	13	7	6	10

* One died of unknown causes.

where the environmental conditions were very severe. However, only the sheep on two of the farms were subsequently shown to have been exposed to natural foci of infection. A histogram of the distribution of antibody titres at the time of vaccination and 4–5 months later is shown in Fig. 1. From this it is apparent that in each group there were more animals with HI titres of 1/160 or greater at the end of 5 months, indicating that sero-conversion continued in individual animals for many weeks after vaccination. This was also the trend in the three sheep retained on tick-free pastures at Moredun Institute.

Of the 36 unvaccinated sheep, 8 died and in 7 of them louping-ill was diagnosed either by recovery of virus or by the presence of neuropathological lesions in the brain, of a severity sufficient to cause death from acute viral encephalitis. However, no deaths occurred on the Perthshire farm and as there was no indication in the

unvaccinated sheep of rise of antibody titre it is evident that only 32 vaccinated and 24 of the unvaccinated sheep in two of the groups were actually exposed to the risk of natural infection. As indicated in Table 3, 7 unvaccinated sheep died of clinical louping-ill, 6 others showed rise of antibody titre and were presumed to be naturally immune, while 10 had no HI antibodies, indicating that they had not encountered infected ticks although exposed to the same risks as their companions. Only 13 of 24 unvaccinated sheep were therefore shown to have circulated louping-ill virus after exposure to natural foci of louping-ill.

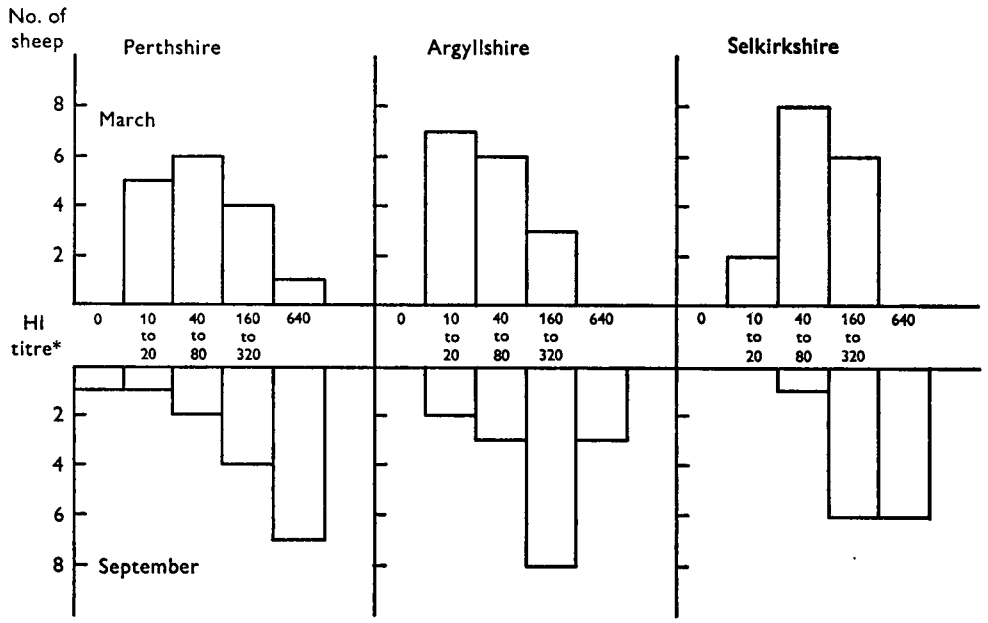

Fig. 1. Distribution of HI titres at vaccination and after 4–5 months.
* Reciprocal serum dilution.

Vaccination of animals recovered from natural infection

On the Argyllshire farm, four of the rams vaccinated in May and one which had not been vaccinated were made available for a blood test in September. In May their HI titres were 1/320, 1/320, 1/40, 1/160 and 1/160; on retest the respective titres were 1/20,480, 1/5120, 1/5120, 1/1280 and 1/160. Vaccination of naturally immune animals had apparently raised the HI titres to a remarkable degree. The last animal, which had not been vaccinated, maintained the same titre throughout the period of 5 months.

Maternal immunity transferred from ewe hoggs vaccinated in March 1969

In Table 4 are recorded the HI titres of the sheep surviving on the Selkirkshire farm and left there until they produced lambs. All of the animals vaccinated in March 1969 still had good immunity when tested 6 months later and again in May 1970 after lambing. Although the tests were conducted at an interval of 6 months

the HI titres of individual animals were in reasonable agreement except that 3K87 and 4K86 showed an 8- to 16-fold rise during the interim.

Of the seven surviving unvaccinated controls, four had no serological evidence of immunity in September and were presumed to be susceptible and three had titres of 1/40, which was judged to be the result of recovery from louping-ill. They

Table 4. *HI titres of ewes and lambs tested in September* 1969 *and May* 1970

Sheep no.	HI titre dams: Sept. 1969	HI titre dams: May 1970	Lambs, May 1970
3K59	40	80	*
3K80	160	320	320
3K82	80	80	160
3K83	160	320	640
3K84	320	320	320
3K85	160	80	160
3K86	160	320	160
3K87	40	640	160
3K88	80	80	160
3K89	20	20	20
4K84	40	20	40
4K85	40	40	20
4K86	160	1280	160
	Vaccinated Oct. 1969		
4K10	0	160	640
4K12	0	80	320
4K13†	40	640	1280, 1280 ‡
4K14	0	1280	—
4K15†	40	> 2560	2560
4K16†	40	> 2560	2560
4K19	0	160	2560, 2560 ‡

* No lamb.
† Immune from natural infection.
‡ Twins.

were all vaccinated and when tested again in the following spring all had responded. Numbers 4K13, 4K15 and 4K16 had unusually high titres consistent with their previous experience of natural infection before vaccination, but 4K14 was higher than the average to be expected in an animal experiencing antigen for the first time.

Two of the 20 sheep did not produce a lamb, 2 had twins and all of the 20 lambs born had very good maternal immunity as judged by HI serum titres when tested between birth and 14 days of age (Table 4).

DISCUSSION

The results of the field trials show that a single dose of inactivated oil-adjuvant vaccine was able to elicit a serologically detectable immune response in sheep, and vaccinated animals apparently acquired complete immunity to louping-ill.

In contrast, 7 out of 24 (29%) of the unvaccinated controls known to have been exposed to a natural virus focus died with clinical and pathological evidence of louping-ill. In addition, vaccinated sheep maintained on one farm for a second season were able to pass on a very substantial maternal immunity to their lambs. Although the number of sheep was small, each trial was well controlled. Very similar results were obtained in those trials conducted simultaneously in the south and in the west of Scotland where the climatic and environmental conditions were very different and where both groups were shown to have been exposed to infection with tick-borne fever within the first few weeks of arrival. In southern Scotland the exposure to TBF and louping-ill was simultaneous, but in the west exposure to louping-ill was delayed in most of the animals until 3 months after they were introduced, and this raises some speculation about the alleged stressing effect of TBF in favouring the invasion of the central nervous system by louping-ill virus. Possibly the TBF strain was more virulent in the south, and it may be that infection with louping-ill can potentiate pre-existing infection with TBF, leading to further lowering of resistance (Macleod & Gordon, 1932; Gordon *et al.* 1962).

The serological status of the vaccinated sheep (Fig. 1) provided no evidence, from a study of individual or average antibody titres, that exposure to natural foci of virus infection between March and October was reponsible for the increased distribution of higher HI antibody titres towards the end of the period of exposure. The antibody titres of the sheep on the Perthshire farm and the three animals retained at Moredun, which were not exposed, showed a similar trend. Why seroconversion increased in the animals in this trial for many weeks after vaccination is not immediately apparent, but it is a feature of the immunity induced by oil-adjuvant louping-ill vaccine which we have observed in earlier experiments. It may be due to the repeated stimulation of the antibody-forming mechanism by excess antigen. Brotherston & Boyce (1970) challenged ten sheep 2 months after vaccination with 10^4 ID50 of virulent virus and failed to show any increase in HI titre or neutralization index to indicate sufficient multiplication of virus to cause an anamnestic response.

The lambs born to the vaccinated sheep left on the Selkirkshire farm all showed evidence of a considerable maternal immunity and, as recorded in Table 4, all the HI titres except three were 1/160 or greater. Although it cannot be claimed that vaccination of the ewes was solely responsible for the efficient transfer of maternal antibodies after 1 year, previous experience (Brotherston & Boyce, 1970) would suggest that this was so in at least a proportion of the sheep. There was a remarkable correlation between the HI antibody detectable in the individual ewe and her lamb and this was particularly evident in 4K13, 4K15 and 4K16, where the serological responses of both were greatly enhanced.

It is recognized, however, that individual sheep vary in their immunological competence to react to an antigenic stimulus and we have found a small proportion of sheep in which HI titres reach a maximum of 1/20 after vaccination and in which the decay of antibody is faster than that of the majority which reach titres of 1/160 or greater. In Table 4 sheep 3K87 and 4K86 are recorded as showing a 16-fold and an 8-fold increase in the titre between tests conducted in September

1969 and May 1970 when compared with those of their fellows. It is suggested that in the intervening period of over a year since first vaccination the immunity in these individuals declined to a point where challenge by virus-infected ticks in the spring of 1970 was responsible for a boost to their immunity. Whether this was accompanied by virus multiplication or was an accelerated response due to previous sensitization is not known at present. Sheep 4K13, 4K15 and 4K16 also showed a greatly enhanced serological response, and this was most likely due to vaccination of animals sensitized by previous challenge rather than exposure to louping-ill virus after vaccination, although the latter cannot be ruled out entirely. However, similar results were also obtained in the immune rams vaccinated on the farm in Argyllshire and this tends to confirm the view that vaccination enhances the serological response induced by natural infection.

Epidemiological features. According to the results shown in Table 3, 13 of 24 sheep exposed to known foci of louping-ill infection circulated virus, and of these about half developed fatal encephalomyelitis and half became immune. The real incidence of clinical disease in sheep which were actually challenged was 7 out of 13, or over 50 %, but it is equally important to remember that about 40 % of the unvaccinated sheep never encountered infected ticks, so that it is reasonable to assume that this also holds good for all the animals exposed to natural foci of infection in these trials.

The idea that vaccine of low immunogenicity, particularly an inactivated one, may be used to control louping-ill by permitting systemic infection but not encephalitis is a novel one, and a similar suggestion was made by Mayer, Blaškovič, Ernek & Libíková (1969), who used a highly attenuated TBE virus before exposing sheep to contact with virulent virus circulating in nature. They suggested that the immunity conferred by immunization may be maintained or even increased by small booster doses of virus transmitted by the ticks and the concept undoubtedly worked well when all animals were subjected to repeated antigenic stimulus.

On the pastures in Argyllshire the autumn-feeding population contained the highest proportion of infected ticks because, despite a heavy spring infestation, only one sheep circulated virus compared with five which became infected 5 months later, but this situation was reversed in the trial in the south of Scotland. Clearly exposure of animals for one season to tick-infected pastures is no guarantee that all of the survivors and their offspring will be immune to infection in succeeding years or that natural foci of virus can be relied upon to regularly maintain or boost a waning immunity to louping-ill as it occurs in Britain.

More information is required about the distribution and characteristics of louping-ill virus transmitted by ticks and why some sheep develop encephalomyelitis and others become immune after systemic infection.

The use of attenuated virus as an immunizing agent poses problems and safeguards of a different nature to those encountered with an inactivated agent and the inactivated vaccine described here offers many advantages for combating infection with louping-ill virus. A single dose provides adequate protection for sheep and their lambs for at least 1 year and very probably for 2 or 3 years (Brotherston, Boyce & Renwick, unpublished) and the preparation, storage and

injection of vaccine are relatively simple. Further experience may reveal that it is possible to incorporate the antigen into other types of vaccine used by sheep farmers routinely each year. This would ensure maximum protection in breeding stock and lambs in the spring and it is likely that the residual maternal immunity would be sufficient to protect lambs from a late focus of infection occurring in autumn.

The regular use of this prophylactic measure may provide the means not only of protecting from clinical disease but also of reducing the transfer of virus from parasitized domestic animals to succeeding stages and generations of *Ixodes ricinus* ticks.

We thank Dr P. C. Doherty for the neuropathological examinations and Mr C. C. Renwick for managing and distributing the sheep flocks. We express our gratitude to Mr J. B. Boyce and Mr C. Burrells for their able assistance with the serology.

REFERENCES

Brambell, F. W. R. (1958). The passive immunity of the young mammal. *Biological Reviews* **33**, 488.

Brotherston, J. G. & Boyce, J. B. (1969). A new vaccine against louping-ill. *Veterinary Record* **84**, 514.

Brotherston, J. G. & Boyce, J. B. (1970). Development of a non-infective protective antigen against louping-ill (Arbovirus Group B). *Journal of Comparative Pathology* **80**, 377.

Doherty, P. C. (1969). Louping-ill encephalitis in C 57 black mice. *Journal of Comparative Pathology* **79**, 571.

Doherty, P. C. & Reid, H. W. (1971). Louping-ill encephalomyelitis in the sheep. II. Distribution of virus and lesions in nervous tissue. *Journal of Comparative Pathology* (in the Press).

Foggie, A. (1951). Studies on the infectious agent of tick-borne fever in sheep. *Journal of Pathology and Bacteriology* **63**, 1.

Gordon, W. S. Brownlee, A., Wilson, D. R. & Macleod, J. (1962). The epizootiology of louping-ill and tick-borne fever with observations on the control of these diseases. *Symposia of the Zoological Society of London*, no. 6, pp. 1–27.

Macleod, J. & Gordon, W. S. (1932). Studies in louping-ill (an encephalomyelitis of sheep). II. Transmission by the sheep ticks *Ixodes ricinus* L. *Journal of Comparative Pathology* **45**, 240.

Mayer, V., Blašovič, D., Ernek, E. & Libíková, J. (1969). The serological response and long-lasting resistance against infection with louping-ill virus in sheep immunized with a highly attenuated tick-borne encephalitis virus. *Journal of Hygiene* **67**, 731.

O'Reilly, K. J., Smith, C. E. G., McMahon, D. A., Bowen, E. T. W. & White, G. (1968). A comparison of measuring the persistence of neutralizing and haemagglutinin-inhibiting antibodies to louping-ill virus in experimentally infected sheep. *Journal of Hygiene* **66**, 217.

Reid, H. W. & Doherty, P. C. (1971). Louping-ill encephalomyelitis in the sheep. I. The relationship of viraemia and the antibody response to susceptibility. *Journal of Comparative Pathology* **81**, 291.

Smith, C. E. G. (1969). Arbovirus vaccines. *British Medical Bulletin* **25**, 142.

Smith, C. E. G., McMahon, D. A., O'Reilly, K. J., Wilson, A. L. & Robertson, J. M. (1964). The epidemiology of louping-ill in Ayrshire: the first year of studies in sheep. *Journal of Hygiene* **62**, 53.

Williams, H. & Thorburn, H. (1961). The serological response of sheep to louping-ill virus. *Journal of Hygiene* **59**, 437.