

STUDIES ON RIFT VALLEY FEVER.—PASSIVE AND ACTIVE
IMMUNITY IN LAMBS

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Smithburn (1949) reported that humoral immunity to Rift Valley fever (R.V.F.), transmitted from an immune ewe to her twin lambs, persisted for about five months and was not affected by weaning. He postulated that this might be of practical value in protecting young lambs during the period of maximum susceptibility to Rift Valley Fever virus.

In the Republic of South Africa, arthropod-borne diseases of sheep such as Rift Valley fever, Wesselsbron disease and bluetongue occur seasonally during the late summer and autumn, and almost invariably disappear soon after the first frosts of winter. Bearing this in mind, practical use is made of transmitted passive immunity to protect the lambs. Autumn lambing is advocated, and farmers are advised to immunise all their sheep during the spring, first with a polyvalent bluetongue vaccine and later with a combined Rift Valley fever-Wesselsbron vaccine.

On account of the abortifacient and embryotropic properties of the neuro-adapted Rift Valley fever and Wesselsbron disease viruses which form the basis of the live attenuated virus vaccine, prophylactic immunisation is restricted to non-pregnant animals. At present, for the preparation of the Rift Valley fever vaccine, Smithburn's neurotropic attenuated strain of virus at the 102nd mouse intracerebral passage level is used.

Mulligan (1937) prepared a similar type of vaccine from a neurotropic strain of virus at the 92nd mouse passage level. He reported that "the vaccine proved safe for lambs six weeks old and produced a good immunity. Tested in the field, on a large number of pregnant ewes and newly born lambs, this vaccine was found to be unsafe for the newly born lambs and to cause abortion in some of the pregnant ewes. Vaccination of pregnant ewes, moreover, did not appear to result in the transfer of the immune bodies through the colostrum to the lamb although, in this connection, it must be stated that lambing had started at the time vaccination was practised and the interval between inoculation and the birth of a lamb was never more than a few days. It became apparent, however, that the vaccine, while it might be of service in protecting maiden ewes, was not suitable for controlling an outbreak in pregnant ewes and very young lambs."

Kaschula (1953) using Smithburn's neurotropic virus at the 86th passage level reported abortions among pregnant ewes and the death of lambs shortly after birth under field conditions in South Africa.

The investigations reported in this communication were undertaken initially to determine the effect of the strain of Rift Valley fever virus incorporated in the vaccine on pregnant ewes and day-old lambs. During the course of the investigations valuable information was obtained on the nature and duration of transmitted immunity in lambs and the immune response of day-old lambs to the neurotropic strain of Rift Valley fever virus.

STUDIES ON RIFT VALLEY FEVER

MATERIALS AND METHODS

Rift Valley fever vaccine.—The vaccine strain of Rift Valley fever virus at the 102nd mouse intracerebral passage was used. The virus, in the form of a 2·5 per cent brain suspension, was freeze-dried in 0·5 ml. amounts. The intracerebral LD₅₀ of the reconstituted virus for adult mice was $10^{-7.5}$ per 0·025 ml. For immunisation of the ewes and lambs a 1/100 dilution in distilled water was used subcutaneously, reconstitution and dilution being carried out immediately before use.

Sheep.—Ten Merino ewes, showing visible signs of pregnancy, were selected for the experiment. In order to prevent the newly born lambs from sucking milk before being bled, the udders of the ewes were enclosed in a leather bag, which was strapped to each animal at the start of the experiment. Each lamb was bled as soon as possible after birth and was then allowed to suck from its mother.

Sera.—Serum samples were collected from each ewe before the start of the experiment, again on the day of parturition and at irregular intervals thereafter. Newly born lambs were bled before they had sucked, again 24 hours after sucking and thereafter at the intervals indicated.

In all cases blood was withdrawn aseptically from the jugular vein and the serum was removed from the clot within eight hours. The sera were stored at -18 C° . Immediately before use in the *in vitro* neutralisation tests, each serum sample was inactivated at 56 C° for 30 minutes.

Neutralisation tests.—Quantitative serum-virus neutralisation tests were carried out by the method described by Weiss (1957). In this procedure, which was carried out at 4 C° , undiluted serum was mixed with falling 10-fold dilutions of virus antigen. After contact for 18 hours at 4 C° , 0·1 ml. of serum-virus mixture was injected intraperitoneally into each of a family (usually six) of day-old white mice from the Onderstepoort closed inbred colony. The mice were kept with their mothers under observation for a period of 14 days. The virus used in the neutralisation tests was the NRVF2 strain, which had had in succession 102 mouse intracerebral, 56 embryonated egg and 16 mouse intracerebral passages (Weiss 1957). The antigen was stored in dry ice as a 10 per cent mouse brain suspension in 10 per cent horse serum saline.

Diluent.—Ten per cent horse serum saline was used as diluent for all the dilutions of virus used in the neutralisation tests.

EXPERIMENTAL PROCEDURE

Each of seven of the ten pregnant ewes received 1·0 ml. Rift Valley fever vaccine subcutaneously. The lambs were born seven to 23 days later and were bled from five minutes to 12 hours after birth without being allowed to suck from their mothers. In the case of lamb No. 7116 the bleeding and first suck were delayed for 24 hours after birth. Each ewe was bled on the day its lamb was born and both ewe and lamb were bled at the intervals shown in Table 1.

The three remaining ewes were not immunised during pregnancy. Within 18 hours of birth the three lambs were bled and were given 1·0 ml. of Rift Valley fever vaccine subcutaneously. Two of the three ewes were also vaccinated at the same time as their lambs. The remaining ewe (No. 2129) was not vaccinated.

The neutralisation indices of the sera of the ewes and lambs collected at the intervals shown are given in Table 1.

TABLE 1.—*Neutralisation index of serum of ewes and lambs immunised with Rift Valley fever live attenuated virus vaccine*

Ewe and Lamb No.	Vaccination data	Neutralisation index of ewes (pre-immunisation) and lambs (pre-suckling)	Neutralisation indices of sera collected after immunisation of ewe or lamb. Time interval in days in parenthesis									
			1-1	2-8	5-0	6-0+	6-0+	6-0+	6-0+	6-0+	6-0+	6-0+
2103	Immunised 7 days before birth of lamb.....	0	1-1 (1)	2-8 (7)	5-0 (18)	6-0+ (24)	6-0+ (29)	5-8+ (38)	4-7 (172)	—	—	—
7113	Lamb not immunised.....	0	4-4 (6)	4-4 (6)	6-0+ (11)	6-0 (22)	4-5 (31)	—	3-3 (165)	—	—	
2112	Immunised 11 days before birth of lamb.....	0-5	0-1 (1)	— (7)	5-2 (11)	5-5 (24)	6-0 (38)	—	—	—	—	
7116	Lamb not immunised.....	0-5	— (1)	— (7)	0-75 (13)	0 (27)	—	—	—	—	—	
2114	Immunised 12 days before birth of lamb.....	0	0-4 (1)	4-1 (12)	6-0+ (18)	—	—	—	—	—	—	
7117	Lamb not immunised.....	0-5	— (1)	Lamb died	6th day	—	—	—	—	—	—	
2171	Immunised 15 days before birth of lamb.....	0-5	4-2 (1)	4-8 (9)	4-3 (15)	4-3 (24)	4-8 (29)	4-8 (34)	4-6 (38)	4-2 (43)	3-2 (172)	
7120	Lamb not immunised.....	4-0	— (1)	— (9)	5-1 (14)	4-2 (23)	1-0 (28)	—	0-2 (38)	—	0 (157)	
2144	Immunised 22 days before birth of lamb.....	0-5	1-5 (1)	4-7 (22)	5-5 (28)	—	—	—	—	—	—	
7125	Lamb not immunised.....	0-4	— (1)	Lamb died	4th day	—	—	—	—	—	—	
3412	Immunised 22 days before birth of lamb.....	0	5-2 (1)	5-1 (22)	5-0 (63)	5-3 (98)	3-3 (133)	4-5 (161)	—	5-5 (202)	5-5 (287)	
5220	Lamb not immunised.....	5-5	— (1)	3-8 (28)	— (63)	4-7 (91)	— (133)	— (161)	3-4 (168)	3-5 (182)	3-4 (264)	
2108	Immunised 23 days before birth of lamb.....	0-5	3-0 (1)	2-9 (6)	2-0 (11)	0-7 (23)	0 (28)	0 (33)	0-3 (38)	0-9 (43)	0 (172)	
7126	Lamb not immunised.....	0	— (1)	— (6)	— (11)	1-8 (20)	1-5 (30)	—	—	—	0-4 (149)	
2187	Immunised the day lamb was born	0	0-3 (5)	1-9 (10)	2-9 (15)	3-1 (25)	4-9 (35)	—	—	—	—	
7105	Lamb immunised at birth.....	0	3-7 (5)	5-4 (10)	5-4 (15)	5-3 (25)	5-8 (35)	3-6 (169)	—	—	—	
2121	Immunised the day lamb was born	0	0-5 (5)	4-4 (10)	4-3 (15)	3-7 (26)	3-7 (30)	—	—	—	—	
7114	Lamb immunised at birth.....	0	— (5)	4-8 (10)	4-7 (16)	4-7 (21)	—	—	—	—	—	
2129	Ewe not immunised.....	0-5	2-7 (8)	4-6 (14)	5-8 (19)	0-6 (28)	28 days after birth of lamb	—	—	—	—	
7115	Lamb immunised at birth.....	0	— (8)	— (14)	— (19)	6-0+ (28)	—	—	—	—	—	

RESULTS AND CONCLUSIONS

There was no clinical manifestation of ill health nor an abortion, among the seven ewes immunised during pregnancy. Two of the animals, No. 2114 and 2144, which were treated 12 and 22 days respectively before parturition, gave birth to weak lambs which died within the first six days of life. On examination both lambs showed lesions of a viral encephalitis, but unfortunately virus isolation was not attempted. This is taken to indicate intra-uterine transmission of virus infection manifested only post-partum.

In the case of ewe No. 2114, which lambed 12 days after immunisation, the neutralisation index of the serum rose from a pre-immunisation titre of 0 to 4·1 at parturition. Her lamb, No. 7117, had no specific antibody to significant titre in its serum, either before or 24 hours after sucking and it died on the sixth day. The neutralisation index of the serum of ewe No. 2144 rose from 0·5 to 4·7 at parturition, 22 days after vaccination. Her lamb, No. 7125, showed a very slight rise in neutralising antibodies to an index of 1·5 24 hours after sucking but died on the fourth day. Both these lambs failed to show the expected rise in antibody titre after sucking, in spite of high titre antibodies in the sera of both ewes at the time of parturition. Both lambs died as a result of encephalitis undoubtedly caused by the neurotropic virus used for immunisation of the ewes. Apparently intra-uterine infection of the foetus with Rift Valley fever virus had taken place, and, though the lambs were born alive, the reaction was not controlled by the ingested colostrum antibody.

The neutralisation tests on the sera of the remaining five ewes vaccinated during pregnancy and on the sera of their lambs, which survived, yielded most interesting results.

Ewe No. 3412 which gave birth to lamb No. 5220, 22 days after vaccination, showed high titre antibody in her serum at the time of parturition and maintained the titre of antibody for more than nine months. The pre-sucking serum of her lamb, taken 15 minutes after birth, had a neutralisation index of 5·5. There was no detectable rise in antibody 24 hours after sucking and the antibody persisted for more than 264 days, which was the longest period tested. The presence of circulating antibody in the lamb immediately after birth and before sucking colostrum milk, together with the persistence of this antibody for at least nine months, indicates that virus infection followed by active immunisation of the foetus had occurred *in utero*.

Ewe No. 2103 showed a significant rise in antibody titre from 0 to an index of 2·8 on the seventh day after immunisation when lamb No. 7113 was born. As would have been expected the antibody titre rose progressively to a titre greater than 6·0 on the twenty-fourth day and was maintained at a high level for 172 days. The pre-sucking serum of the lamb, No. 7113, showed no detectable antibody. A slight rise in the neutralisation index to 1·1 was apparent 24 hours after sucking and this increased gradually to an index of more than 6·0 by the eleventh day after sucking. At 165 days (approximately 5½ months) the neutralisation index of the serum had declined to 3·3. It is impossible to be dogmatic in regard to the origin of the antibody in this particular lamb. The absence of serum antibody immediately after birth, and the acquisition of low titre antibody after colostrum ingestion from a ewe showing at that time only low titre antibody, support the hypothesis that the

antibody was derived from the colostrum. This contention, however, cannot explain the rise in antibody titre up to the eleventh day and the maintenance of a relatively high titre up to the one hundred and sixty-fifth day. A more feasible explanation would appear to be intra-uterine infection with vaccine virus resulting in active immunisation, whether or not that reaction was controlled or modified by the ingested colostrum antibody. In this connection it must be borne in mind that the pregnant ewe received its vaccine injection only seven days before parturition so that there was insufficient time for the foetus to develop an active immunity prior to birth, as was the case with lamb No. 5220.

Ewe No. 2112, which gave birth to lamb No. 7116 eleven days after immunisation, had developed high titre serum antibody at the time of parturition. The lamb, prevented from sucking for at least 24 hours, at no stage showed the presence of serum antibody in significant titre. It is concluded that transmission of passive immunity to this lamb failed owing to the delay in the intake of colostrum. Although there is no direct evidence that antibody was still present in the colostrum when the lamb had its first suck, there is every reason to believe that it has not disappeared.

The serum of ewe No. 2171, immunised 15 days before parturition, developed a serum neutralisation index greater than 4.0. The serum of its lamb No. 7120 had a neutralisation index of 4.0 immediately after birth and before the lamb had access to colostrum milk. This high index was maintained for 23 days, after which it dropped suddenly to 1.0 on the twenty-eighth day and disappeared soon thereafter. The relatively high titre antibody in the pre-suck serum and the fact that the antibodies persisted for only a short period of time indicate that diaplacental transfer of antibodies occurred in this particular case, in addition to possible colostrum immunity, to produce a transient passive immunity.

Ewe No. 2108 which was immunised 23 days before birth of lamb No. 7126 failed to develop a significant rise in antibody titre. The lamb, which showed no antibody in its pre-sucking serum, acquired a low grade passive immunity demonstrable 24 hours after sucking. The immunity gradually declined until only a trace of antibody was detectable 30 days after its first suck. The only possible explanation is that although the ewe failed to develop the anticipated high titre serum antibody there was nevertheless a sufficient concentration in the colostrum to confer on the lamb a low titre passive immunity of short duration.

The neurotropic virus produced no untoward effects on the three susceptible lambs out of non-immune ewes immunised immediately after birth. The three lambs No. 7105, 7114 and 7115 responded by developing high titre antibodies in their sera. By comparing the immune responses of the lambs with those of the two ewes immunised simultaneously it can be seen that the lambs developed a serum neutralisation index greater than that of the adult sheep and within a much shorter period of time. To illustrate the point the following figures are abstracted from the table: The neutralisation index of the serum of ewe No. 2187 increased from 0 to 0.3 at five days, 1.9 at ten days, 2.9 at 15 days, 3.9 at 20 days and 4.9 at 35 days after immunisation, whereas in the case of her lamb No. 7105, the neutralisation index increased to 3.7 at five days, 5.4 at ten days and 6.0 at 20 days after immunisation. It is therefore apparent that lambs from susceptible ewes may be actively immunised against Rift Valley fever within 24 hours of birth, with safety.

DISCUSSION

The number of animals in this experiment is far too small to warrant any generalisations but certain positive results were obtained which appear to be irrefutable and are of considerable academic as well as practical importance.

Experience with the immunisation of many thousands of adult sheep in the field has shown that this live attenuated virus vaccine is quite innocuous when administered to mature animals. In addition, the immunity produced appears to have been effective in that mortality on subsequent exposure to natural virulent infection has not been observed. This field observation is confirmed by the finding that eight out of nine ewes developed high titre serum antibody by approximately the eleventh day after treatment. On the other hand one ewe failed to respond by the development of persistent high grade immunity. Therefore, some breakdowns in the field during an enzootic must be anticipated, though the vaccine itself is safe.

The response of the two lambs out of susceptible ewes to vaccine administered immediately after birth occasioned some surprise in view of Mulligan's experience (1937). Neither lamb showed any detectable clinical reaction and rapidly developed high titre serum antibodies indicative of active immunisation. This finding supports the hitherto doubted contention of breeders that in the face of an enzootic lambs may be immunised at birth with impunity.

Among seven pregnant ewes immunised between the seventh and twenty-third days before parturition there were no abortions but two lambs died within six days of birth. There would appear to be no doubt that infection developed *in utero*. It is reasonable to conclude that, had a larger number of ewes been treated and particularly at an earlier stage of pregnancy some deaths of the foetuses followed by either abortion, foetal mummification or absorption would have occurred. The important point is that, except in the face of an enzootic, immunisation of susceptible pregnant ewes cannot be advocated and if resorted to, may be anticipated to affect the lamb crop adversely. This is supported by findings in the field.

A point of very considerable academic importance is the finding that although the foetus may be infected with the attenuated vaccine virus *in utero* death of the foetus does not invariably follow and that an active durable immunity initiated *in utero* may be completed either pre- or post-partum. This did in fact occur in one of the seven lambs born.

It is believed that it has been established that the neurotropic attenuated virus of Rift Valley fever may infect the foetus carried by a pregnant susceptible ewe. The ultimate outcome of this infection probably is dependent on several factors, the most important being the stage of pregnancy of the ewe. The foetus may die *in utero*. The foetus may be carried to full term and die shortly after parturition. On the other hand the foetus may be actively immunised *in utero* thus being born with high titre antibody in the serum, not connected with diaplacental transfer of antibody nor colostral antibody from sucking within the first 24 hours of birth.

The development of active immunity by a foetus *in utero* is an entirely new finding, apparently not previously recorded. It is contrary to the general belief that the human infant and the new born animal is capable only of low grade antibody response (Burnet, 1955). According to Burnet it is possible that an antigen introduced sufficiently early in life will become a "self" constituent and will not provoke an antibody response in the host. If this hypothesis is correct then it would appear that in sheep the state of non-reactivity or tolerance to Rift Valley fever virus is limited to very early foetal life.

SUMMARY

1. The neurotropic virus of Rift Valley fever at the one hundred and second intracerebral mouse passage level may be used for the production of a safe vaccine for the active immunisation of adult Merino sheep.

2. There is some evidence to show that the same vaccine is equally innocuous and effective when used on lambs from susceptible ewes even on the day of birth.

3. Immunisation of pregnant susceptible ewes cannot be advocated because it has been shown that intra-uterine infection of the foetus does take place, the outcome of such infection being—

(a) death of the foetus with or without abortion (previous field observations);

(b) apparently normal parturition but followed by death of the lamb within the first few days of birth;

(c) birth of an actively immunised lamb—an apparently new observation.

4. There has again been demonstrated the transfer of antibody from immune ewe to lamb by ingestion of colostrum but it is important that the first suck should not be delayed for as long as 24 hours after birth.

5. In one case there was evidence of intra-uterine transfer of serum antibody from dam to foetus.

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