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## STUDIES ON TRANSMISSION OF MAEDI VIRUS TO LAMBS\*

By

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SIHVONEN, LIISA: *Studies on transmission of maedi virus to lambs.* Acta vet. scand. 1980, 21, 689—698. — Lambs born to 5 ewes in 3 successive years were studied for presence of maedi virus and its antibodies. In the middle of the first-year pregnancies the ewes and the only ram of the colony were inoculated with maedi virus. No antibodies or viraemia could be detected in the lambs at birth. After sucking colostrum, antibodies appeared in the lambs of the ewes which themselves were seropositive, and reached their peak in a few days. Maternal antibodies disappeared within 12 weeks in all the lambs. Neutralizing antibodies were demonstrated in the colostrum and their content declined rapidly after lambing. Virus was isolated from the milk of 2 ewes in the third year of the study.

In the first year the spread of maedi virus was demonstrated to only 1 of the lambs, but in the other 2 years maedi virus was detected in tissues of half of the lambs sacrificed at 3—12 weeks of age. It was concluded that lambs born to chronically infected ewes are readily infected, indicating excretion of virus by ewes. The study yielded no information on the specific routes of transmission, except for the finding of the virus in milk of 2 ewes in the third year of the study. No evidence was obtained of transplacental transmission of maedi.

maedi virus; transmission of virus; maternal antibodies.

Icelandic experience (*Pálsson 1976*) indicated that maedi was most likely spread naturally as a droplet infection while the sheep were in close contact. Maedi has been transmitted experimentally also by administering faeces from diseased to healthy sheep in drinking water. An indirect spread of maedi, however, is considered to be exceptional (*Pálsson*).

Lambs born and reared by ewes suffering from maedi often become affected with maedi (*Pálsson*). The presence of maedi virus in milk from affected ewes 1—5 months after lambing has been demonstrated in zwoegerziekte (*de Boer 1970*).

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Attempts to demonstrate transplacental transmission of maedi yielded negative results in Iceland and the Netherlands (*Gudnadottir* 1974 and *de Boer & Houwers* 1979). In Denmark, however, maedi virus was isolated from 3 lambs delivered by caesarian section close to term from infected ewes and thereafter reared in isolation for 8—9 months (*Hoff-Jørgensen* 1977).

This paper reports on studies on the transmission of maedi virus to lambs in an experimentally infected sheep colony. Some of the data on viraemia and immune responses in the ewes of the colony have been published earlier (*Sihvonen et al.* 1980a,b, *Sihvonen* in press).

#### MATERIALS AND METHODS

The lambing history of the ewes is described in Table 1. Six ewes, of which 1, No. 1296, was a Texel sheep, the other 5 Finnish sheep, were mated with ram No. 14 in the first study year. All the sheep were free of infectious diseases and healthy. In mid-gestation 2 ewes (Nos. 10 and 11) were inoculated via the respiratory tract and 2 (Nos. 1296 and 1360) intracerebrally with maedi virus. At the same time sheep No. 1008, not pregnant in the first year, was inoculated via the respiratory tract and ram No. 14 received the first dose of the hyperimmunizing series of virus inoculations. The inoculation of the sheep has been described earlier (*Sihvonen et al.* 1980a). Two sheep (Nos. 15 and 16) served as uninoculated controls. The maedi-inoculated ewes were again mated in the following 2 autumns with the ram. All the experimental sheep were in the same isolation unit which was divided into smaller parts with walls when required. All the sheep were in close contact with each other. There was 1 clinical case of maedi in a Texel sheep kept together with the sheep of the present study during the first study year (*Sihvonen et al.* 1980a).

The lambs, 29 in all, were sampled for viraemia at birth and thereafter the lambs of the first year every 2 months, the lambs of the second year every month for 4 months and then every 2 months and the lambs of the third year every 2 weeks for 2 months and thereafter every month by examining the blood leukocytes with the tissue culture techniques previously described (*Sihvonen et al.* 1980a).

Sera were collected from the lambs at birth and every week thereafter and tested for antibodies. The methods for comple-

ment fixation (CF) and neutralization tests (SN) have been described earlier (*Sihvonen et al.* 1980b).

The lambs were sacrificed at varying times after birth and examined for virus isolation and histopathological changes as described earlier (*Sihvonen et al.* 1980a). Five lambs are still under observation.

The lambs of the first and second study year were weaned at the age of 6—8 weeks and the lambs of the third study year at the age of 3½—4 months.

Blood for sera was collected from each ewe every week and blood leukocytes were examined for viraemia every month for 16 months from inoculation and thereafter every 2 months. Milk was collected in the second study year from each ewe twice, at lambing and 3 weeks later, and in the third study year 12 times, about every week for 3—4 months, and examined for virus and antibodies. The milk and separated cells of the milk were examined for virus in sheep choroid plexus cell cultures as described for blood leukocytes. The neutralization test for antibodies in milk was carried out as for serum antibodies (*Sihvonen et al.* 1980a).

Table 1. Three-year lambing history of 5 ewes inoculated in mid-gestation of the first study year with maedi virus and the fate of their lambs.

Ewe No.	Lamb No.		
	Study year		
	1	2	3
10	24 <sup>b</sup>	29 <sup>b</sup>	41 <sup>c</sup>
	25 <sup>b</sup>	30 <sup>c</sup>	42 <sup>b</sup>
11	17 <sup>b</sup>	27 <sup>b</sup>	43 <sup>a</sup>
	18 <sup>b</sup>	28 <sup>b</sup>	44 <sup>b</sup>
1296	20 <sup>b</sup>	33 <sup>b</sup>	45 <sup>a</sup>
1360	21 <sup>a</sup>	31 <sup>b</sup>	50 <sup>c</sup>
	22 <sup>b</sup>	32 <sup>b</sup>	46 <sup>a</sup>
	23 <sup>c</sup>	34 <sup>b</sup>	47 <sup>c</sup>
1008	*	35 <sup>b</sup>	48 <sup>a</sup>
15**	26 <sup>b</sup>	*	49 <sup>b</sup>
16**	19 <sup>b</sup>	*	*

<sup>a</sup> Sacrificed 0—6 h after birth.

<sup>b</sup> Sacrificed 3 weeks to 1 year after birth.

<sup>c</sup> Still living.

\* Not mated.

\*\* Uninoculated controls.

## RESULTS

*Occurrence of antibodies*

At birth before sucking colostrum none of the 11 lambs serologically studied had antibodies. After sucking colostrum antibodies appeared in 14 out of 25 lambs and reached their peak within the first days. Their ewes showed antibodies at lambing. The maternal antibodies disappeared within 12 weeks in all the lambs (Table 2). The 11 negative lambs were born to negative ewes except for No. 18 in the first study year when no maternal antibodies could be demonstrated though the ewe had low levels of antibodies like the twin lamb. It is emphasized that no neutralizing antibodies were found in the lambs of the first year, but they were demonstrated in the following 2 years. So far no antibodies have been detected in the sera of the lambs after the disappearance of maternal antibodies.

The occurrence of neutralizing antibodies in colostrum and in the subsequent milk of the ewes in the third year is shown in Table 3. Closely corresponding titres of serum and colostrum and the rapid disappearance of antibodies from the milk were noted.

*Virus isolation*

Two attempts, the first at lambing and the second 3 weeks after lambing revealed no maedi virus in the milk of the ewes in the second-year trials. Maedi virus could be isolated from cells of the milk of ewes Nos. 10 (once: at 2 months after lambing) and 1360 (twice: at 3 and 5 weeks after lambing) in the third year when the 5 sheep were sampled 12 times within 4 months of lambing.

The occurrence of viraemia in ewes is shown in Table 4 and in lambs in Table 5. No viraemia could be detected in any lambs from birth to the age of 11 weeks. Viraemia could be detected only in 1 first-year lamb (No. 23) in 2 out of 10 attempts carried out at the age of 11—26 months. Viraemia could be detected in 1 lamb (No. 31) of the second year at the age of 11 weeks and during the 6—12 month period in another lamb (No. 32) in 4 out of 10 trials. The other second-year lambs were negative for viraemia for 1 year, but in the only surviving lamb (No. 30) viraemia was detected subsequently once at the age of 13 months. The detection of viraemia was negative for lambs of the third year during the first 4 months.

Table 2. Occurrence of maternal neutralizing (SN) and complement fixing (CF) antibodies in selected lambs and the titre in their respective ewes at lambing.

Study Lamb year No.	Titre at												Titre at lambing								
	0 h		6 h		2 days		1 week		3 weeks		5 weeks		7 weeks		9 weeks		12 weeks		Ewe No.	CF	SN
	CF	SN	CF	SN	CF	SN	CF	SN	CF	SN	CF	SN	CF	SN	CF	SN	CF	SN			
1	17	NT	NT	NT	NT	NT	64	0	32	0	16	0	0	0	0	0	0	0	11	32	0
	24	NT	NT	32	0	32	0	16	0	0	0	0	0	0	0	0	0	0	10	32	0
	25	NT	NT	32	0	32	0	16	0	0	0	0	0	0	0	0	0	0	10	32	0
2	27	NT	32	16	256	128	256	128	256	128	64	64	32	8	32	8	8*	0	11	128	256
	28	NT	32	16	512	128	64	64	128	64	32	32	16	8	16	8	0	0	11	128	256
	30	0	0	NT	128	16	128	16	64	8	32	8	32	8	16	8	0	0	10	128	16
3	41	0	0	NT	64	16	128	64	64	32	32	8	32	8	0	0	0	0	10	128	32
	44	NT	512	256	NT	256	128	128	64*	0	0	0	0	0	0	0	0	0	11	512	256
	49	0	0	NT	32	8	32	8	16	8	0	0*	0	0	0	0	0	0	1008	32	16

NT = Not tested.

\* Time of sacrifice.

Table 3. Neutralizing antibody titres to maedi virus in sera and milk of the 5 ewes in the third study year.

Ewe No.	Titre in serum at lambing	Titre in milk days after lambing		
		0	4	7-9
10	64	32	0	0
11	256	256	16	0
1008	16	16	0	0
1296	0	0	0	0
1360	0	0	0	0

The results of virus isolation trials from sacrificed lambs are shown in Table 6. Virus was isolated most commonly and roughly at an equal rate from the plexus choroidalis, lungs and spleen. The earliest isolations were made from lambs sacrificed at the age of 7 weeks in the second year and at 5 weeks in the third year. No maedi virus could be isolated from the 5 lambs sacrificed shortly after birth. One of them (No. 45) had sucked

Table 4. Occurrence of viraemia in the 5 ewes in the 3 study years.

Study year	Viraemia detected in ewes Nos.				
	10	11	1008	1296	1360
1	11/12*	10/12	10/12	4/12	2/12
2	3/8	5/8	4/8	4/8	4/11
3**	2/4	1/4	2/4	0/4	2/4

\* Number of positive attempts / total number of attempts.

\*\* Results for 6 months only.

Table 5. Occurrence of viraemia in lambs born in the 3 study years.

Study year	Time after birth			
	0-2 months	2-4 months	4-12 months	12 months
1	0(0)/19(10)*	0(0)/7(5)	1(1)/25(5)	1(1)/9(1)
2	0(0)/18(9)	1(1)/14(5)	2(1)/25(4)	1(1)/2(1)
3	0(0)/24(10)	0(0)/9(3)		

\* Number of virus isolations (number of positive lambs)/number of isolation attempts (number of lambs).

Table 6. Isolation of maedi virus from tissues of sacrificed lambs.

Age of lamb at sacrifice	Number of lambs with positive isolation/total number tested			
	Study year			Total
	1	2	3	
0—6 h	0/1	NT	0/4	0/5
3—5 weeks	NT	NT	1/3	1/3
7—12 „	0/3	3/5		3/8
4—12 months	0/5	2/3		2/8

colostrum for 6 h, the other 4 had received no colostrum. Second-year lamb No. 28 was sacrificed at 1 year and then had an almost full-born lamb in uterus. Virus was isolated from the ewe but not from its foetus. None of the lambs sacrificed within 1 year of birth had any histopathological changes suggestive of maedi.

#### DISCUSSION

The role of maternal antibodies to maedi virus has not been clarified and they may well have no significant protective function. This does not affect the fact and pattern of their existence. No data on the subject have been published earlier. The present results indicate that the maternal antibodies disappeared in all the lambs within 3 months. The titre and duration corresponded, as expected, with the titre of antibodies in the serum and colostrum of the respective ewes. The data seem to fit the general pattern of the occurrence of maternal antibodies in sheep.

The antibody content in milk declined quite rapidly within a few days of lambing. The kinetics agree with that of disappearance of some other colostral antibodies (*Wells 1978*) and do not indicate any local maedi infection in the udder.

No evidence of transplacental transmission of maedi virus was obtained in the present studies. This agrees with the fact that no maedi virus was found in 30 fetuses of maedi-affected ewes in the Netherlands by *de Boer & Houwers (1979)*. However, *Hoff-Jørgensen (1977)* isolated maedi virus from 3 lambs delivered close to term from infected dams by caesarian section. The significance of the finding is somewhat obscured by the fact that the isolation was made from lambs kept for 8—9 months.

The results of virus isolation trials bear more directly on the

subject of transmission of the infection from ewes to lambs. Virus isolation attempts from ewes' milk twice within 3 weeks after lambing in the second study year were negative. During the third study year when milk samples were taken 12 times for 3—4 months, maedi virus could be detected in 2 ewes' milk and not earlier than 3 weeks after lambing. *De Boer* (1970) reported the recovery of maedi virus from the milk of ewes 1—5 months after lambing in the Netherlands. He did not state whether negative attempts were made during the first month. The ewes of this experiment had detectable viraemia irregularly in the second and third study years. The rare detection of the virus from milk may well be correlated with this more general fluctuation.

Nevertheless, in general, the results suggest that lambs born to infected ewes will readily become infected. Proximity to and dependence upon the ewes obviously facilitate the transmission. The results of the experiments support epidemiological field findings (*de Boer & Houwers* 1979).

An apparent difference in infectivity of the cases of infection was demonstrated in the present studies. Maedi virus was isolated from tissues of 3 out of 5 7—12-week old lambs of the second study year and in 1 out of 3 3—5-week old lambs of the third study year when most of the lambs were sacrificed shortly after birth. Isolations were made also of older second-year lambs. It can be concluded that the group of chronically infected ewes in the second and probably also in the third study year was an effective source of infection to the lambs. The result of the first year differed. These lambs did not become infected or at least virus could not be isolated from their tissues or blood during the study period. The exception was the lamb kept for long term observation and found viraemic not earlier than at the age of 9 months. It seems evident that the transmission of the virus by the ewes was ineffective during the first months of their infection. Evidence that infectivity and viraemia are not closely correlated is the fact that the ewes were frequently demonstrated to be viraemic already during the first study year.

In contrast to frequent isolations of virus from tissues, detection of viraemia in lambs was quite rare. Virus was isolated, for example, from 4 sacrificed lambs that had never been detected viraemic. The discrepancy might be due to the low level of circulating virus and relative insensitivity of the test used for the detection of viraemia.



The present study yielded no data on the specific routes of transmission, except for the finding of the virus in 2 ewes' milk in the third study year which indicates the possibility of milk-borne infection. Various routes of direct contact may be possible. The subject requires further study. Indirect transmission is less probable on epidemiological grounds.

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## SAMMANFATTNING

*Undersökningar angående överföringen av maedi virus till lamm.*

Lamm födda av fem tackor under tre på varanda följande år undersöktes med avseende på förekomsten av maedi virus och dess antikroppar. Efter halva dräktighetstiden under det första året inokulerades tackorna och den enda baggen i gruppen med maedi virus. Inga antikroppar eller viremi kunde påvisas hos lammen vid födelsen. Efter att ha fått kolostrum kunde antikroppar påvisas hos lamm till de tackor som själva var seropositiva. Antikroppstitern hos lammen nådde sitt maximum inom några dagar. De maternala antikropparna försvann inom 12 veckor hos alla lammen. Neutraliserande antikrop-

par kunde påvisas i kolostrum och deras kvantitet sjönk snabbt efter lammningen. Virus isolerades från mjölken av två tackor under det tredje året.

Under det första året kunde en spridning av maedi virus påvisas hos bara ett lamm, men under de två därpå följande åren kunde maedi virus redan påvisas i vävnaderna hos hälften av lammen som avlivades i en ålder av 3—12 veckor. Man kan sluta sig till att lamm födda av kroniskt infekterade tackor lätt kan bli infekterade, vilket tyder på att tackorna utsöndrar virus. Beträffande de specifika överföringsvägarna kunde relevanta fakta inte erhållas i detta arbete, med undantag av upptäckten av virus i mjölken hos två tackor under det tredje året. Inga bevis för en transplacental transmission av maedi kunde erhållas.

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