Chapter 5

SRLV: Transmission I

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Comparative quantification of the lateral transmission of Maedi Visna virus in two flocks of sheep of different genetic constitution.

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Abstract
A comparative prospective study in two flocks of sheep of different genetic constitution quantified the rate of natural lateral transmission of Maedi Visna virus (MVV). Transmission was monitored by means of detection of specific antibodies and PCR-detection of the viral genome in whole blood. Both tests appeared to be of similar sensitivity in early detection of infection. The recorded rate of transmission at pasture was very low with between 0.12 and 0.19 new infections per infected ewe per year. However, during the short housing period, which simulated the usual indoor lambing season, the transmission rate increased to between 2.27 and 3.4 new infections per infected ewe per year. The differences between the flocks were statistically non-significant. Viral transmission appeared to cease at a certain point in time, which supports the notion that individual differences in susceptibility to lateral infection occur. The results of this study give rise to new views on the prevention, and new options for the control, of MVV-infections.

Key words: Maedi Visna Virus / SRLV / Sheep / lateral transmission / susceptibility

Introduction

Maedi Visna Virus (MVV) of sheep is a single-stranded RNA virus belonging to the group of Small Ruminant Lentiviruses (SRLV's) which causes chronic lymphocytic lesions in multiple organs after relatively long incubation periods. It is transmitted horizontally either by means of ingestion of infected colostrum and milk -lactogenic transmission- or by direct transmission between animals -lateral transmission- [1]. Particularly, the intimate contact between the ewe and her offspring appears to be important [2, 3]. Vertical -prenatal- transmission does occur, but its contribution to the spread of the infection is generally considered limited [1, 4].

Ever since MVV was established as a specific viral disease, a breed associated difference in susceptibility to infection and the subsequent development of disease has been apparent. Probably the most poignant example of this were the healthy Karakul rams imported into Iceland in 1933 which introduced a disastrous disease in the native sheep population [5, 6] whereas there are records over many years that strongly suggest that the original Karakul population in Germany never showed symptoms of disease suggestive of MVV [7]. So far, two studies suggested individual differences in susceptibility to MVV [8, 9] and several studies indicated that certain sheep breeds were less susceptible [3, 10, 11]. The results of these studies cannot be compared because they were influenced by several, typically local, variables like the keeping conditions and the management applied (stocking rate, selection/replacement policy etc) and, particularly, if there were indoor lambing periods. So, comparative studies are required to actually prove the notion of breed susceptibility.

In longitudinal epidemiological studies, particularly those focusing of the dynamics of the transmission, serology as a means to detect infection has the drawback of the relatively slow and individually variable antibody formation after infection, which makes determining the actual point in time of the transmission difficult. It is generally assumed that this can be overcome by using sensitive virus detection methods, e.g. PCR [12].

In order to be able to calculate the risk of transmission under in- and outdoor conditions, e.g. in the framework of prevention and control efforts, basic quantitative data on the transmission rate is required. At the same time this data would give insight in the influence of the conditions on the transmission rate. Such data is currently lacking.

In order to obtain quantitative data on the lateral transmission of MVV and to comparatively study differences between breeds, a prospective study of one year in two cohorts of barren sheep of different genetic constitution on the same farm with a short housing period was performed, using a serological test as well as a PCR-test to detect infection in blood samples taken with short intervals.

Materials and methods

Animals and set-up

Two flocks of 30 ewes each were formed. Flock A consisted of Texel-type sheep and flock B of a relatively new composite breed which was originally drawn from F1 crosses between purebred Texel and purebred Milksheep. The Milksheep breed was almost extinct some four decades ago and genotyping of the current population showed the typical characteristics of an island population, i.e. a high level of inbreeding (personal communication dr J.A. Lenstra). The composite breed drew attention because there were some flocks suffering from MVV-disease to a degree never seen before, i.e. clinical symptoms occurred already in relatively young sheep and particularly arthritis which is very rare in the common Dutch sheep population which mainly consists of Texel-type sheep. The average age of the ewes was 4,5 years ranging from 2,5 to 6 years. Both experimental flocks consisted of 20 MVV-free and 10 infected (seropositive and PCR-positive, see below) barren ewes and were separately kept on pasture on the Veterinary Faculty's farm and were housed separately for 2 weeks to simulate the usual housing period during the lambing season. The stable measured 90 m^2 and had natural ventilation. Hygienic protocols were implemented for all attendants. The ewes did not lamb and were not mated during the study. No concentrates were fed except a handful when luring them into the pens for blood sampling. Early in the study two ewes of Flock B (1 of the infected ewes and one non-infected) died of unrelated causes. The study was approved by the Institutional Ethics Committee on Animal Experiments (license DEC 0311.0501).

Serology and PCR

Every two weeks blood was collected from the jugular vein with a vacutainer system using 10 ml glass EDTA-tubes (BD Vacutainer Systems, Preanalytical Solutions, Plymouth, UK) . Whole blood and separated plasma were stored at – 20 ^{0}C within 24 hours. Specific antibodies were detected with Elitest-MVV (HYPHEN BioMed, Andrésy, France) according to the manufacturers' instructions. This test was previously described [13] and extensively compared with other tests (Brinkhof et al, submitted for publication**).**

For PCR, DNA was extracted from 100 µl whole blood using the ABI PRISM 6100 Nucleic Acid PrepStation (Applied Biosystems) with its proprietary blood chemistry including the proteinase K treatment. Extracted whole blood DNA was subjected to real-time PCR using primers directed at the Long Terminal Repeat (LTR) [14]. Amplification was performed with a LightCycler 1.5 instrument (Roche Diagnostics GmbH, Mannheim, Germany) using SYBRgreen as reporter agent and melting point analysis. The reaction mix consisted of 10 µl PCR mix (QIAGEN's Quantitect SYBR Green 1), 1 µl of each primer (12.5 pmol/µl), 2 µl sample DNA and 6 µl sterile ultrapure water. PCR procedure started with 15 min at 95°C –hot start- followed by 45 amplification cycles of 20 sec at 65°C, 15 sec at 72°C and 15 sec at 95°C.

Statistical comparison of test results
The results of PCR and Elitest-MVV were compared by a generalised linear mixed model using a binomial error distribution and a logit link function. The result of Elitest-MVV was the dependent variable in the model (1 = positive, 0 = negative) and the result of the PCR (1 = positive, 0 = negative) the explanatory variable. To take into account the interdependence of test results from the same animal, we included animal as a random effect in the model (this interdependence makes comparison by Cohen's Kappa invalid). Moreover, to examine if breed influenced the performance of the tests, we also included the interaction between breed and test result in the model.

Analysis of the transmission
On the basis of the outcome of experimental infection studies [15-17], we assumed for the analysis that antibodies against MVV infection could be detected 4-8 weeks after infection. In addition, we assumed that sheep were infectious immediately after they had been infected and that they remained infectious for the rest of the experiment. Next, if $\lambda(t)$ is taken as the rate of infection for an susceptible individual, then the probability that a randomly assigned susceptible animal that was non-infected at time t has become infected at time $t + dt$ equals

$$\lambda(t)dt + o(dt) \qquad \text{(a)}$$

From eq. (a) can be derived [18] that the probability that a randomly assigned susceptible animal that was non-infected at t1 has become infected at t2 equals

$$1 - \exp(-\int_{t1}^{t2} \lambda(t)dt) \qquad \text{(b)}$$

Based on Eq. (b), a binomial model can be formulated for the number of new infections (C) between t1 and t2 (C(t1,t2)):

$$C(t1,t2) \approx Binom.(S(t1);1 - \exp(-\int_{t1}^{t2} \lambda(t)dt)) \qquad \text{(c)}$$

with S(t1) is the number of susceptible animals at $t1$ and

$$\lambda(t) = \beta \frac{I(t)}{N(t)} \qquad \text{(d)}$$

the rate at which a susceptible individual becomes infected, in which β is the transmission rate parameter (number of new infections caused by one infected sheep per unit of time in a fully susceptible population), I(t) is the number of infected sheep at t and N(t) the total number of sheep at t.
We quantified β by a Generalized Linear Model (GLM), using a binomial error distribution [19]. In this model, the number of new infections in an interval was the dependent variable and breed and housing (outside or inside) were included as explanatory variables. Because of the uncertainty of the time from infection to seroconversion (see above), the housing period was included in the analysis as the

period from four weeks before the housing until four weeks after. The random and systematic components of the model were linked by a complementary log-log function and the average fraction of infected sheep (*I/N*) multiplied by the number of days in the interval was included as an offset variable. As a consequence, the outcome of the model is the number of new infections per infectious sheep per unit of time. The fit of the statistical model was checked by the mean residual deviance and by plotting standardized residuals against predicted values.

Results

Serology and PCR

The results of the serological test and the LTR-PCR are presented in a two by two table (Table I); PCR has fewer positive test results than serology. However, the analysis showed a strong association between both tests. The odd of a seropositive test result giving a positive result in the PCR is 16.9 times (95% CI 6.0-47.9) as high in comparison to the odds of a seropositive test result giving a negative PCR. Although there was a trend for a stronger association between both tests in Flock B, the interaction between breed and test result was not significant ($p = 0.08$).

Serology and LTR-PCR were equally efficient at early detection, i.e. both tests turned positive at virtually the same point in time. However, post conversion, PCR gave a less consistent result over time, particularly in a few specific animals in flock A (not shown).

Two by two table				
	PCR			
		Neg	Pos	Tot
Serology	Neg	167	12	179
	Pos	41	146	187
	Tot	208	158	366

Table I: Two by two table of the results of the serology and the LTR-PCR on whole blood of the sequential samples from 14 sheep that naturally acquired Maedi Visna Virus infection during the sampling period.

Lateral transmission

In Flock B the first new infection was detected in week 18, whereas in Flock A this occurred in week 23. Overall, 9 ewes in Flock B were newly infected during the year, whereas in Flock A only 5 ewes got infected, as determined by serology as well as PCR. Moreover, transmission seemed to extinguish after a certain period of time; 31 weeks in Flock B and 34 weeks in Flock A (Fig 1 and 2).

The transmission modelling showed that the transmission rate was significantly higher (p = 0.0049) during the period of housing, i.e. 3.4 infections per infectious ewe per year in Flock B and 2.27 infections per infected ewe per year in Flock A, as compared to the outdoor period when the transmission rates were 0.19 and 0.12, respectively (Table II). However, although the transmission rate in Flock B, both in- and outdoors, appeared higher than in Flock A, these differences were not statistically significant (p = 0.43).

Flock B

Week / Ewe	0-12	12	15	18	20	21	22	23	24	26	28	30	31	32	35	37	39-49
1	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+
2	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+
3	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+
4	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+
5	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+
6	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+
7	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+	+
8	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+
9	-	-	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+
10-19	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
20	†																
21-29	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
30	†																
Total	28	28	28	28	28	28	28	28	28	28	28	28	28	28	28	28	28
MVV +	9	9	9	12	12	12	13	13	13	13	14	15	18	18	18	18	18

Figure 1: Lateral transmission pattern of Maedi Visna Virus infection under field conditions during one year, as determined with serology (Elitest-MVV; HYPHEN BioMed, Andrésy, France) in Flock B. The vertical bold lines depict the housing period (week 18 and 19) and an estimated period (8 weeks post infection) for seroconversion related to the housing.

Flock A																	
Week / Ewe	0-12	12	15	18	19	20	21	23	25	28	30	32	34	36	38	40	41-50
1	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+	+	+
2	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+
3	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+
4	-	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+
5	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+
6-20	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
21-30	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Total	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30
MVV +	10	10	10	10	10	10	10	11	11	12	13	13	15	15	15	15	15

Figure 2: Lateral transmission pattern of Maedi Visna Virus infection under field conditions during one year, as determined by serology (Elitest-MVV; HYPHEN BioMed, Andrésy, France) in Flock A. The vertical bold lines include the housing period (week 20 and 21) and an estimated period (8 weeks post infection) for seroconversion related to the housing.

Conditions	Breed	New cases per infected animal per year	95% CI
At pasture	Flock a	0,12	(0,015-0,99)
	Flock b	0,19	(0,023-1,50)
Housed	Flock a	2,27	(0,29-18,0)
	Flock b	3,40	(0,43-47,3)

Table II: Number of laterally transmitted Maedi Visna Virus infections per infected ewe per year in two separated flocks on one farm at pasture and during a housing period simulating an indoor lambing season.

Discussion

The methods to detect MVV-infection in this study -serological test and a real-time PCR with LTR-primers in whole blood- gave relatively consistent results. The somewhat limited agreement between both tests is clearly due to a few animals in flock A where PCR-results were less consistent over time. Nevertheless, both methods detected infection at virtually the same point in time which is contrary to our expectation and that of others [12] that PCR would detect earlier. This can be considered as lack of sensitivity, e.g. as a consequence of using whole blood in a plain sample preparation method. However, virtually all the positive DNA preparations sustained 10-fold dilution (results not shown). In addition, other

studies using the same LTR-primers, but also other primers, using separated blood cells and more elaborate preparation methods also showed limited diagnostic sensitivity compared with serology [14, 20]. In this view it is suggested that both serology and LTR-PCR require a certain level of viral replication in the host to become positive which contends that the diagnostic sensitivities of both assays should be in the same range.
In conclusion, the results of the LTR-PCR as applied on whole blood confirmed the results of the serology in this study. The results also demonstrated that most infected animals continuously have provirus carrying monocytes in their circulation, which supports the assumption in the modeling that infected animals are continuously infectious to others.

The calculation of the overall rate of lateral transmission of MVV in the two flocks suggests, but does not prove, a difference in the efficiency of lateral transmission between the breeds. This means that this comparative study did not establish the epidemiological importance of the flocks' genetic constitution, although the observed tendency is in line with the expectation that sheep of Flock B would be more susceptible.
The modeling shows that the lateral spread of the infection was significantly influenced by the short housing period. At pasture, it took an infected ewe in both flocks between 2 and 3 years to infect another one. In conjunction with the average life expectancy of an ewe of four to five years this basically implies that the infection would have faded out if the sheep had not been housed. Note that the sheep were shortly penned for blood sampling every two weeks. However, indoors the efficiency of transmission was totally different; here it took an infected ewe only between 1.2 and 1.9 months to laterally infect another one, which means that the transmission rate was more than tenfold higher during the housing period. It should be noted that the housing conditions were relatively good; a roomy stable with good natural ventilation. Overall, these results suggest that in conditions without housing, MVV-infections will only spread very slowly, if at all, and will probably not reach levels of economic significance, which is in line with recent studies in Spain [20]. However, there is an established epidemiological relationship between the dam and her progeny [3] and if flock management –unknowingly- selects for the progeny of infected dams, MVV-infection rate may increase even without a housing period.
A recent study on lambs born indoors provided data indicating that lactogenic transmission was not as efficient as hitherto contended and consequently that lateral transmission at early age was more important [2]. Results of the current study quantified that lateral transmission between housed sheep can indeed be relatively efficient, which basically corroborates with that outcome. The epidemiological importance of lactogenic transmission is relative to the rate of lateral transmission, which as this study showed, strongly depends on housing. In conclusion, this study quantified that housing is the critical event in the spread of MVV. The generally experienced difficulties with the control of Caprine Arthritis

Encephalitis Virus (CAEV)-infections in modern goat dairies with year-round housing suggest that this conclusion applies for both MVV and CAEV.
An interesting finding was the apparent extinction of lateral transmission at a certain point in time in both flocks. Long standing experience with testing flocks in the field for MVV-infection showed that the initial infection incidences per flock greatly vary, even among flocks of the same breed held under similar conditions and management. Similar observations have been made in other countries. It is suggested that this variation is not reflecting a dynamic process, but a relatively steady situation as the consequence of differences in the overall susceptibility to MVV-infection of the flocks tested. It should be noted that the genetic composition of an established flock only changes slowly in time, but that rams, because of the number of progeny they produce, may have a profound influence in time by introducing susceptibility genes. So, the extinction of transmission documented in this study together with field observations, suggest the existence of individual differences in susceptibility to MVV, which is in line with another recent report [9]. In this view it is hypothesized that the apparent differences between breeds are not breed characteristics, but due to the prevalence of susceptible individuals within the breed. This prevalence is accidental since intentional breeding for resistance has, as yet, not been applied.
Apart from environmental and host factors, SRLV-transmission is probably also influenced by viral factors. In natural transmission studies, however, host and virus cannot be separated and therefore the possible influence of the virus strain cannot be ruled out. So this study employed the natural situation with the viruses that were common to the breeds involved. The fact that sheep seroconverted and became blood-PCR-positive in both flocks and that they had specific lesions at necropsy (to be published elsewhere) proves that the strains involved were at least replicated, which is likely prerequisite for transmission.
In conclusion, this study quantified the lateral transmission of MVV in adult sheep for the first time and demonstrated that housing is a critical event herein. For the prevention and control of MVV this means that management systems without housing run little risk of reaching the infection rates that cause economic damage, which implies that preventative measures should relate to the risk associated with the -mainly geographically determined- management system. This corroborates with the fact that the incidence of MVV in regional sheep populations varies considerably. The recorded cease of transmission in both flocks indicates -genetic- differences in susceptibility to MVV-infection between the individuals in the flock. This theoretically offers the option of controlling MVV in a flock or population simply by decreasing the average susceptibility through structurally selecting the annual replacements from the progeny of the seronegative ewes in an infected flock.

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