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Investigation of risk factors associated with infections caused by small ruminant lentiviruses

Nuria Barquero, Esperanza Gomez-Lucia, Alvaro Arjona, Cristina Toural, Alfonso Las Heras, José F. Fernández-Garayzábal, José Antonio Ruiz-Santa Quiteria, Ana Doménech

Departamento Sanidad Animal,
Facultad de Veterinaria, Universidad Complutense de Madrid; 28040 Madrid, Spain
nuria.barquero@vet.ucm.es

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Abstract

An epidemiological study was conducted to identify risk factors related to small ruminant lentivirus (SRLV) infection in the central region of Spain. Between October 1998 and October 2000, a total of 194 sheep from 10 flocks and 163 goats from three flocks were tested for SRLV antibodies, resulting in 65.5% and 8.0% of seroprevalence, respectively. The relationship between differences in prevalence of SRLV, geographical location of the flock, and possible factors related to the flock that could enhance transmission were studied. Results of multivariable analysis showed an association between SRLV infection and geographical location of the flock and the rearing system. In addition, the differences in the productivity between infected and non-infected animals were explored. The productivity parameters were measured in 62 sheep and 28 goats. All productivity parameters studied (milk production, number of milking days, and lambing rate) appeared to be reduced in the SRLV-seropositive group in both goats and sheep. Even though, these differences were not statistically significant, it seems that animals infected are less productive than these non-infected. Statistical analyses comparing infected and non-infected sheep showed no statistical relationship between SRLV infection and milk quality.

Key words: sheep, goats, small ruminant lentiviruses, risk factors, epidemiology, Spain.

Introduction

Maedi-visna (MV) is an ovine chronic disease, caused by the retrovirus maedi-visna virus (MVV), which was initially isolated in 1964 (24). The disease is characterised by respiratory, nervous, joint, and/or mammary clinical signs. MVV together with caprine arthritis and encephalitis virus (CAEV) are grouped as small ruminant lentiviruses (SRLV), due to their genomic and antigenic similarities.

SRLV are widespread worldwide except Iceland, Australia, and New Zealand (22). Seroprevalence varies between and within countries and studies. The seroprevalence in the USA and Canada is 28% of sheep and 61% of the flocks (1). In Spain, it has been reported that between 12% and 66% of the animals and 30% and 100% of the flocks are infected, but prevalence varies according to the region (3, 20).

Transmission is related to body fluids, mainly lung secretions (aerosol droplet infection), as well as milk and colostrum (17) and it may occur within animals of the same flock, or from mother to offspring (4). Other routes, as venereal and transplacental transmission, have not been entirely demonstrated (4, 21).

The diagnosis of SRLV is based on clinical signs, pathological lesions, and laboratory testing. However, clinical signs are not specific and the infection may be asymptomatic. Consequently, antibody and viral detection are indicated for early diagnosis (22). The OIE recommended the use of either agar gel immuno-diffussion (AGID) or enzyme-linked immunosorbent assay (ELISA). Milk as substrate for the ELISA has been demonstrated as an alternative to blood in several studies (2, 5, 16), and its use simplifies the collection of samples.

Investigations of risk factors associated with the disease have become important in order to establish control measures that could reduce the incidence of the infection. Some studies found that SRLV infection was related to flock size, weaning age, or days that sheep were housed (10, 20). In addition, other authors related the incidence of the infection with housing time,

stocking density, shed ventilation, and age (13). Previous studies conducted by the same authors showed that MVV seroprevalence was also related to the breed and the production system (12).

The aim of the study was to investigate several risk factors related to the management of the flock, which could enhance the transmission, and to explore whether SRLV-infection is associated with differences in the productivity and milk quality.

Material and Methods

Animals and sampling. A total of 194 sheep from ten flocks and 163 goats from three flocks were sampled between October 1998 and October 2000. Farms were located in the central region of Spain (provinces of Toledo, Guadalajara, and Madrid). Milk samples were aseptically collected in 10 mL containers after discarding the first squirt. All the samples were kept at 4°C during transportation to the laboratory (11). The breeds of the sheep included in the study were Assaf (51%), reared in intensive flocks; Manchega (36.6%), in semi-intensive system; and F1 cross breed between them (12.4%), reared in intensive production system (considered as a reference group). The sheep were either mechanically milked (22.6%) or by hand (77.3%) (considered as a reference group). Assaf animals were located mainly in Madrid, Manchega sheep were located in Toledo, Madrid and Guadalajara, and F1 cross breeds were mainly located in Guadalajara. Five flocks were of Manchega breed, four flocks were of Assaf breed, and one flock was of F1 cross breed. Goats were Murciano-granadina breed reared in intensive system and mechanically milked.

SRLV diagnosis. Milk samples were used for the diagnosis of SRLV. A procedure, which is supported by the results of different studies, was applied (2, 5, 16). The commercial ELISA (Elitest Hyphen-Biomed, France) was used for the diagnosis (23). The plates were coated with a combination of the major core protein p25 of MVV produced in *Escherichia coli* and a peptide derived from the immunodominant region of the viral transmembrane protein gp46 (23). Non-centrifuged milk was diluted 1:10 and the analysis was performed following the manufacturers' instructions.

Case definition. All animals positive by the ELISA were considered infected with the virus and denominated as SRLV-seropositive (cases). Animals that had negative ELISA results were classified as non-infected (controls). The percentage of seropositive animals in the flock was considered as seroprevalence of the flock.

Variables examined. The variables included were classified into two categories: variables related to productivity and milk quality, and variables related to the flock and production system.

Variables related to productivity and milk quality. The information about the productivity parameters and milk quality was gathered from 62 Assaf sheep and from 28 Murciano-granadina goats intensively reared. The variables related to productivity included lambing rate (number of offspring born per gestating animal, defined as a continuous variable), milk production (measured in litres), and duration of milking (measured in days).

The somatic cells counts, fat, protein, lactose, and dry extract content were determined in milk in the Lactological Institute of Lecumberri (Navarra, Spain), using azidiol as preserver. The analysis was done in a Combifoss, Fossomatic 250/360 (P.E./ALVO/03) and Milkoscan 255/605 (P.E./ALVO/02), certified by ENAC, at 40ºC 24-48 h after sampling.

Variables related to the flock and production system. The information related to the flock management was gathered from 194 sheep included in the study. The variables of geographical location, breed, production system, and milking technique were analysed. These variables are stated above.

Statistical methods. The statistical analyses were performed using Stata software (Version 8.0 Stata Corp, College Station, USA). Univariable screening of all variables was performed to evaluate the strength of their association with the outcome of SRLV infection, with P-values indicating the probability that any detected differences had occurred by chance alone. For binary and categorical variables chi-squared or Fisher's exact tests for contingency Tables were used as appropriate. The continuous variables were evaluated using the parametric Student's *t*-test.

Logistic regression. Dependent variables were also examined for their univariable association with SRLV infection by the use of single-variable, ordinary logistic regression analysis with results expressed as unadjusted odds ratios (OR), and corresponding 95% confidence intervals and P-values.

Forward stepwise multivariable ordinary logistic regression analysis with flock as a random effect was subsequently conducted to provide valid estimates of the strength of association between them and SRLV infection, after controlling the effects of the other variables included in the regression models. Variables were retained if they were significantly associated with SRLV infection as measured by the Wald $\chi 2$ test ($P \leq 0.05$), or if their inclusion significantly improved the fit of the model (likelihood ratio statistic $\chi 2$ test, $P \leq 0.05$). Effect modification between variables in the final model was investigated, where possible by inclusion of biologically meaningful, two-way interaction terms, which were retained if they provided a significant improvement to model, fit as measured by statistic probability ratio ($P \leq 0.05$).

Results

Out of the 194 animals from 10 flocks included in the study, 127 were considered as SRLV-positive based

on the ELISA results in milk (65.5% seroprevalence). The seroprevalence in each flock varied between 10.0% and 92.3%, and all flocks tested were positive to SRLV. The seroprevalence distribution per breed was 82.88% in Assaf breed reared in intensive flocks; 50% in F1 cross breed reared in intensive production system, and 46.5% in Manchega breed in semi-intensive system. In Madrid, 77.8% of the animals tested against SRLV were positive, 40% of the sheep were positive in Guadalajara, and 47.8% in Toledo. Out of the 163 goats analysed, 13 were found to be positive by ELISA (8.0% seroprevalence). In the case of sheep, all flocks tested were positive to SRLV, and the seroprevalence ranged between 5.2% and 57.1%.

Effect of SRLV infection on milk quality and animal productivity. The results of the analysis are shown in Table 1. According to these results, there was no statistical relationship between SRLV infection and variables related to milk quality. In addition, the findings did not show any statistical relationship between the infection and factors related to animal productivity. However, all the productivity parameters appeared to be reduced in the SRLV-positive group for both sheep and goats. In the SRLV-positive sheep group, the average milk yield was 341.09 litres/ lactation, *vs.* 396.81 litres/lactation in the SRLV-negative group. The prolificacy was 1.34 lambs in seropositive animals *versus* 1.62 in the non-infected group. Finally, the number of milking days was also reduced in the seropositive animals (182.02 d *vs.* 191.63 d) (Table 1). In the goats, the productivity was also reduced. In the SRLV-positive goat group, the average milk yield was 620.67 L/lactation, *vs.* 651.92 L/lactation in the SRLV-negative group. The prolificacy was 1.66 in seropositive animals *vs.* 1.82 in the non-infected group. The number of milking days was 259.16 d in the seropositive goats *vs.* 254.67 d in the seronegative goats) (Table 2). This lack of statistical significance could be related with the low number of animals sampled for these variables.

Risk factors associated with SRLV infection in sheep. Results of univariable logistic regression analysis are summarised in Table 2. The highest percentage of seropositive animals were found in Madrid (77.8%), compared to Toledo (47.8%), and Guadalajara (40.0%). A higher seroprevalence was observed in the Assaf breed, reared in intensive system (82.8%), than in the Manchega, which was reared in semi-intensive system (46.5%), or F1 cross breed, also reared in intensive farms (50.0%). Finally, SRLV-infection was associated with mechanical milking (73.3% seroprevalence *vs* 38.6% in sheep milked by hand).

Table 1. Univariable logistic regression analyses for variables related to milk quality and productivity, means of exposure variables for SRLV infected (positive) and non-infected (negative) groups. The parameters correspond to data from the 62 Assaf sheep included in the study

Variable	ELISA	Mean	Standard Deviation	95% CI[a]	OR[b]	95% CI[c]	P-value
Sheep							
Milk fat	Negative	8.49	1.77	7.69–9.30	reference		
	Positive	14.76	68.74	1.25–28.26	1.00	0.98–1.03	0.752
Dry extract content	Negative	10.37	0.53	10.13–10.61	reference		
	Positive	10.19	1.27	9.94–10.44	0.85	0.51–1.40	0.516
Protein	Negative	4.84	0.68	4.53–5.16	reference		
	Positive	4.97	0.71	4.83–5.11	1.32	0.63–2.78	0.456
Lactose	Negative	4.62	0.45	4.41–4.83	reference		
	Positive	4.61	0.72	4.27–4.55	0.52	0.19–1.43	0.206
Somatic cells count	Negative	1203.62	600.99	158.56–2248.68	reference		
	Positive	1361.30	202.72	959.16–1763.45	1.00	0.99–1.00	0.751
Month of lactation	Negative	2.36	1.21	1.55–3.17	reference		
	Positive	2.29	1.32	1.94–2.64	0.96	0.59–1.55	0.868
Number of lactations	Negative	2.7	1.34	1.74–3.66	reference		
	Positive	3.5	1.64	3.04–3.96	1.48	0.84–2.61	0.171
Milk yield	Negative	396.81	254.35	923.05–1286.95	reference		
	Positive	341.09	367.04	840.69–1049.31	0.99	0.99–1.00	0.212
Lambing rate	Negative	1.62	0.52	1.19–2.06	reference		
	Positive	1.34	0.62	1.15–1.54	0.45	0.12–1.65	0.230
Number of milking days	Negative	191.63	28.51	172.48–210.79	reference		
	Positive	182.02	48.80	165.62–197.43	0.99	0.97–1.01	0.586

[a] Confidence interval for the mean
[b] Odds ratio
[c] Confidence interval for the OR

Table 2. Univariable logistic regression analyses for variables related to milk quality, and productivity, means of exposure variables for SRLV infected (positive) and non-infected (negative) groups. The parameters correspond to data from the 28 goats included in the study

Variable	ELISA	Mean	Standard Deviation	95% CI[a]	OR[b]	95% CI[c]	P-value
Goats							
Milk fat	Negative	4.99	0.73	4.69–5.29	reference		
	Positive	4.18	0.97	1.77–6.59	0.25	0.044–1.42	0.118
Dry extract content	Negative	14.22	0.93	13.83–14.59	reference		
	Positive	13.55	1.11	10.79–16.30	0.49	0.14–1.69	0.259
Protein	Negative	3.76	0.41	3.59–3.92	reference		
	Positive	3.67	0.22	3.14–4.21	0.58	0.03–12.86	0.732
Somatic cells count	Negative	1622.18	1416.72	867.27–2377.10	reference		
	Positive	2327.75	1967.99	1077.34–3578.16	1.09	0.99–1.00	0.274
Number of lactations	Negative	3.60	1.89	2.82–4.38	reference		
	Positive	3.67	0.58	2.23–5.10	1.02	0.52–2.02	0.951
Milk production	Negative	651.92	238.95	553.29–750.55	reference		
	Positive	620.67	132.19	292.28–949.04	0.99	0.99–1.00	0.820
Lambing rate	Negative	1.82	0.33	1.74–2.02	reference		
	Positive	1.66	0.58	0.23–3.10	0.27	0.02–4.00	0.343
Number of days milking	Negative	259.16	39.98	242.65–275.66	reference		
	Positive	254.67	41.78	150.85–358.47	0.99	0.97–1.03	0.850

[a] Confidence interval for the mean
[b] Odds ratio
[c] Confidence Interval for the OR

Table 3. Univariable logistic regression analyses for variables related to the flock and production system, number and percentage of each category of exposure variables for SRLV-infected (ELISA-positive) and non-infected (ELISA-negative) groups. Data correspond to 194 sheep included in the study

Variable	Category	ELISA-negative (%)	ELISA-positive (%)	OR[a]	95% CI[b]	P-value
Province	Guadalajara	27(60%)	18(40%)	reference		
	Madrid	28(22.2%)	98(77.8%)	5.25	2.53–10.89	<0.001
	Toledo	12(52.2%)	11(47.8%)	1.38	0.50–3.78	0.538
Breed	Assaf	17(17.2%)	82(82.8%)	reference		
	F1 cross breed	12(50.0%)	12(50.0%)	0.21	0.08–0.53	0.001
	Manchega	38(53.5%)	33(46.5%)	0.18	0.09–0.36	<0.001
Production system	Semi-intensive	38(53.5%)	33(46.5%)	reference		
	Intensive	29(23.6)	94(76.4%)	3.73	1.99–6.97	<0.001
Milking system	By hand	27(61.4%)	17(38.6%)	reference		
	Mechanical	40(26.7%)	110(73.3%)	4.37	2.15–8.86	<0.001

[a] Odds ratio
[b] Confidence interval

Table 4. Multivariable logistic regression with flock as a random effect including odds ratio, 96% confidence interval and P-value for each category of the explanatory variables. Data corresponds to 194 sheep included in the study

Variable	Category	OR[a]	95% CI[b]	P-value
Province	Guadalajara	reference		
	Madrid	4.26	1.06–17.08	0.041
	Toledo	1.90	0.28–12.63	0.508
Production system	Semi-intensive	reference		
	Intensive	2.60	0.70–9.72	0.012
				0.04*

[a] Odds ratio
[b] Confidence interval
*P value for flock-level random effect term

In the final multivariable logistic regression model milking type and breed became non-significant, whereas, production system and geographical location were the variables that remained in the model (Tables 3 and 4).

Discussion

As far as it is known, this is the first epidemiological study conducted in the central region of Spain that explores risk factors associated with SRLVs infection. In addition, the results also measure the impact of the infection with SRLV on the productivity of goats and sheep in Spain.

No significant evidence on the variation of the milk composition and milk production was observed, which is in agreement with findings of other authors (15). The impact of SRLV-infection on the productivity presented in other studies is controversial; some studies showed a reduction of the productivity (8, 19), while in other studies no effect was detected (9, 15), or no association between MVV infection and lambing rate was found (1, 9). In goats, several studies reported no association between CAEV infection and reduction of productivity and milk quality (18, 27). Other studies found a reduction of the milk yield in goats in the first lactation, but these differences disappeared in subsequent lactations (14). In this study, all productivity parameters measured appeared to be reduced in the seropositive groups for both goats and sheep, even though the differences were not statistically significant. It is possible that the small sample size makes to assess these differences difficult as the data of productivity parameters only belonged to 62 sheep and 28 goats. However, it seems that the tendency of the infection is to decrease productivity, and more studies involving a larger number of animals are necessary in order to formally assess whether there is a decrease in the productivity of animals infected by SRLV.

An association between the seroprevalence and geographical location was observed in the study. These results were not surprising because seroprevalence varies between, and within countries and studies. In Spain, it has been reported that between 12% and 66% of the animals, and between 30% and 100% of the flocks are infected by MVV (3, 20). The evidence of geographical variation of the infection was also observed in England (7). However, it is possible that the apparent geographical association with SRLV infection could be related to management factors, which have not been included in the study.

The association between the rearing system and SRLV infection found in the study was statistically significant. The relationship between seroprevalence of SRLV infection and the production system has been described previously. It was found higher in intensively reared sheep than in semi-intensively and extensively reared sheep (12, 20). The seroprevalence found in our study in intensively-reared Assaf (82.8%) was similar to the 77% observed in previous studies in intensively-reared Assaf sheep (12). In addition the seroprevalence in semi-intensively reared Manchega sheep (46.5%) was also very similar to that reported for semi-intensively reared Rasa Aragonesa sheep (52.8%) in the region of Aragon (North-Eastern Spain) (20). Our results support the previous findings stating that transmission and infection are favoured by the close contact of animals in flocks intensively reared (12).

Some studies have suggested a breed susceptibility to MVV infection (6, 7, 26), while others found no association between both factors (25). In this study, the breed was not a significant factor in the multivariable analysis. However, each breed was kept in a different production system and it was not possible to determine how much effect in the seroprevalence of SRLV was related to the breed, and how much was related to the production system. The results presented here demonstrate that the seroprevalence of the cross-breed reared intensively was lower than the seroprevalence of Assaf intensively reared. On the other hand, the seroprevalence in Manchega breed semi-intensively reared was higher than the seroprevalence in Latxa sheep semi-intensively reared (25%) as reported in other studies (12). Factors such as the number of infected animals that constituted the initial flock, animal density, and occurrence of reinfections may explain these findings. The viral strain, breed differences, and production system could also contribute to the SRLV infection.

In conclusion, it is possible that the small number of samples makes to assess these differences difficult as the data of productivity parameters only belonged to 62 sheep and 28 goats. However, it seems that the tendency of the infection is to decrease productivity, and more studies involving a large number of animals are necessary in order to formally assess whether there is a decrease in the productivity of animals infected by SRLV. In addition, although the number of samples of both animals and flocks was small, and further studies with larger samples are needed, the study indicates that SRLV-infection is strongly associated with the rearing system, which could be related to the close contact of the animals, which enhances transmission of the infection.

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