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Small ruminant lentiviruses in goats in southern Italy: Serological evidence, risk factors and implementation of control programs

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ABSTRACT

Small ruminant lentiviruses (SRLVs) can drastically affect milk production in goat flocks and only an early detection can control and prevent their spread. Since SRLVs are responsible for persistent infections, antibody screening is the most valuable tool to identify infected animals. ELISA is recommended as the election test both for its sensitivity and for its ability to detect low antibody titers, thus identifying infected animals earlier than agar gel immunodiffusion (AGID). In the present study, an investigation was conducted to assess the SRLV seroprevalence in goat flocks in southern Italy and a transversal comparative study was carried out through the analysis of the possible risk factors influencing SRLV spread. A total of 4800 sera from 1060 flocks were analyzed and overall seroprevalences of 18,64% and 51,69% at animal and herd levels, respectively, were observed. Both the region and the herd production systems were able to affect seroprevalence, differently from the herd size, probably because the mean number of goats per herd is low and the semi-intensive management is similar regardless of the dimensional class of each herd. In particular, meat producing herds showed the higher seroprevalence, as a result of the poor sanitation and low animal monitoring in comparison to milk producing herds, where animals are managed twice daily and the relationship between dams and kids is checked to guarantee an adequate quantitative/qualitative milk yield. In the absence of vaccines or effective treatments, health preventive management and seroepidemiological investigations are the only successful approach to restrict SRLV spread as observed in countries were official/voluntary control programs are carried out.

1. Introduction

Caprine arthritis encephalitis virus (CAEV) and Maedi Visna virus (MVV) are currently referred to as small ruminant lentiviruses (SRLVs) due their phylogenetic correlation and the interspecies transmission between goats and sheep. SRLV, a member of the genus *Lentivirus* of the *Retroviridae* family (subfamily *Orthoretrovirinae*), represents a serious economic threat to small ruminant farming, which has led to the development of control programs all over the world.

Genetic variability is a trait of SRLV genome: the emergence of new field isolates in different geographical areas, is a consequence of the virus quasi-species caused by mutations, recombinations and selection pressure by the host immune system. Early phylogenetic studies have suggested that SRLV can be divided into five genetic groups, A to E, that differ from each other in 25–37% of their nucleotide sequences, with A and B further subdivided in subtypes A1–A15 and B1–B3, respectively (Minguijón et al., 2015). Virus isolates from goats in Norway belong to group C, those from Switzerland and Spain are representative of group D (Gjerset et al., 2006; Glaria et al., 2009), while group E is divided into two subtypes, E1 and E2, from northern Italy and Sardinia, respectively (Grego et al., 2009; Reina et al., 2010; Bertolotti et al., 2013;). Subtypes A5 and A7, as well as groups C and D have been isolated only in goats, while subtypes A2 seem to circulate only in sheep. Subtypes A1, A3, A4, A6, B1 and B2 have been found in both species (Olech et al., 2012).

CAEV is largely diffused in dairy goats in most industrialized countries, but it is rarely found in the indigenous breeds of developing areas unless they have had contact with imported goats. Virus transmission usually occurs early in life from infected dams to their kids by the ingestion of virus-containing colostrum or milk. CAEV infects goats for life, but most infected animals remain asymptomatic, though both asymptomatic and symptomatic animals can shed and transmit the virus during all their life and the unapparent carriers represent an important and potential source of infection. The initial clinical signs in kids may include lameness, ataxia, hind limb placing deficits,

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hypertonia and hyperreflexia, albeit they appear bright, alert, and continue to eat and drink normally. The neurologic signs gradually worsen to paraparesis, tetraparesis or paralysis. Affected kids are either euthanized for welfare/economic reasons or eventually die of secondary causes such as pneumonia or exposure. Neurological signs are rarely reported in adults. Chronic, painful polyarthritis (the carpal joints are most often affected), accompanied by synovitis and bursitis, is the main syndrome in adult goats. Indurative mastitis with progressive decrease in normal-appearing milk production is evident, often associated with agalactia at parturition. It is evident that the diffusion of SRLV infection in the herd can significantly affect milk production. Overall, milk production is estimated to decrease by 10% in affected herds (Martínez-Navalón et al., 2013). Occasionally, goats with serologic evidence of CAEV infection may develop chronic interstitial pneumonia and progressive dyspnea (Barrero Domínguez et al., 2017). In the absence of vaccines or effective treatment, health preventive management remains the only approach to limit the spread of SRLV, thus reducing the prevalence of infection. These prophylactic measures include: i) separation of kids from seropositive dams immediately at birth; ii) use of heat-treated colostrum or CAEV-negative colostrum; iii) increase of culling rate; and iv) periodic serological surveillance and immediate segregation of seropositive goats. In nation-wide eradication programs, quarantine of infected herds aids the final stages of a control program.

In Italy, a record growth rate was observed for the national production of goat milk that, between 2010–2016, registered a 162% increase, going from 11,840 to 31,000 tons, relaunching goat breading as a profitable livestock activity. Nevertheless, few studies have described SRLV prevalence, the risk factors associated to SRLV infection in goat population and the negative impact on performance parameters such as decrease in milk production and milk quality (Gufler et al., 2008; Tavella et al., 2018). In this study, the current seroprevalence of SRLV among goat flocks in southern Italy was evaluated and a transversal comparative study was carried out through the analysis of the possible risk factors influencing SRLV spread.

2. Materials and methods

2.1. Study plan and sampling

The goat population of two large regions of southern Italy where small-ruminants breeding is one of the most important livestock activities, consists of 202,125 animals (60,085 in Apulia and 142,040 in Calabria) distributed in 5682 flocks (1439 in Apulia and 4243 in Calabria) (data upload on 12/31/2017, National Reference Center). A total of 1060 goat herds were tested from January 2016 to January 2018. Veterinary officers of the National Health System (NHS) visited flocks and collected a total of 4800 blood samples from the selected herds during the compulsory, official eradication and surveillance programs on brucellosis of small ruminants, for which sheep and goats must be periodically sampled. Sera were separated at laboratories of the NHS and aliquots were sent to our laboratories for SRLV serological testing. No further blood sampling was carried out. Animal sampling was approved by the Ethical Committee of the Department of Veterinary Medicine, Bari, Italy (prot. n. 1153-III/13). On the day of sampling, goat farmers answered a questionnaire prepared by the authors to get information on the last 5 years, regarding flocks and individual animals: gender, age, breed, origin (purchased or born in the flock), number of goats in the flock, health status, vaccination history, management (breeding, dairy or meat attitude), housing conditions, introduction of goats from other herds, and feeding.

The age-categories sampled were productive animals starting from the age of 1 year, to avoid misinterpretations due to the potential presence of maternally derived antibodies. After centrifugation, serum samples were stored at −20 °C until serological testing.

2.2. Enzyme-linked immunosorbent assay

Detection of antibodies directed against SRLV in individual caprine serum samples was performed using the enzyme immunoassay MVV/CAEV p28 Ab verification (IDEXX Laboratories, Inc, USA), following the manufacturer's instructions. The test is an indirect ELISA based on the use of an immunogenic peptide of a transmembrane protein (TM, env gene) and of the recombinant p28 protein, which enters into the composition of the viral capsid (gag gene). The appearance of anti-p28 antibodies can occur slightly later than that of the anti-viral envelope protein antibodies. The use of the very stable protein p28 allows the serological detection of a wide range of SRLV variants. The antibody test kit reported a sensitivity (Se) of 97.9% and a specificity (Sp) ≥ 99.8%.

Microplates were coated in alternating with control antigen (-Ag) and MVV/CAEV antigen (+Ag). Samples to be tested were dispensed in duplicate in the appropriate –Ag well and in the appropriate + Ag well. Upon incubation of the test samples, any antibody specific for MVV/CAEV bound to the antigen in the + Ag wells and formed antigen-antibody immune complexes on the plate well surface. Reading of optical density (OD) was performed using microplate photometer (Microplate Reader, Model 680, Biorad Laboratories Inc.) at 450 nm. The amount of specific antibodies (NE) value for each sample (S) was calculated by subtracting the corresponding OD value obtained in the Control well (S_{-Ag}) from the OD value obtained in the coated well (S_{+Ag}). The result was obtained by comparing the NE of each sample with the mean NE of the positive control (NEX_{pc}). Interpretation of the OD results was obtained calculating the sample to positive ratio (S/P) for each sample by this formula: $100x(NE/NEX_{pc})$. Sera were considered positive when the ratio S/P was higher than or equal to 120%. Doubtful results were recorded when S/P ranged between 110% and 120%. Samples with an S/P ≤ 110% were considered negative.

2.3. Statistical analysis

To include different areas of the regions, the herds were selected through a stratified sample proportional to the province distribution of herds, with a random selection in each stratum. The analysis was performed with the sample size calculator (Creative Research System, Sebastopol, USA). The sample size has been calculated setting a confidence interval of 2.73% and 1.4% for herds and animals respectively, a confidence level of 0.95, and the number of herds and animals as scheduled by the national database. Besides, the number of calculated goats has been distributed among each herd in proportion to its dimensional class (Thrusfield, 2004). The data set was subjected to Chi-quadro test in order to compare incidence of serology positivity between geographic areas (Apulia vs. Calabria), different herd size (< 100 vs. > 100) and different production (meat vs. milk/meat). Moreover, variables were submitted to multivariate logistic regression in order to determine their association power and to calculate Odds ratio (OR) (confidence level 95%, CI_{95}) to confirm their role as risk factors.

3. Results

Though the questionnaire addressed to the farmers the possible risk factors were elaborated, and a transversal comparative study was carried out from the serological results and from the information obtained during the visit in the flocks. Goat breeding in southern Italy is mainly semi-intensive, no kidding area is planned in the flocks, the reproductive system is based exclusively on natural mating and no regular and systematic cleaning and disinfection programs are planned. Consequently, seroprevalence was determined according to two relevant risk factors: size herd and type of breeding, beyond the region factor.

During the observation period, a total of 4800 goat sera were

Table 1
SRLV seroprevalence (%) and confidence interval (CI_{95}%) according to geographic area, herd size and herd specialization.

	Analysed	Positives	Seroprevalence	CI_{95} (%)	P value
Geographic area					
Apulia	268	160	59.70	53.82-65.57	< 0.05
Calabria	792	388	48.99	45.50-52.47	
Herd size					
< 100	412	207	50.24	45.41-55.07	n.s.
> 100	231	126	54.54	48.12-60.96	
Production					
Meat	244	145	59.42	53.26-65.58	< 0.001
Milk/Meat	303	131	43.23	37.65-48.81	

collected from 1060 flocks and analyzed for SRLV antibody detection. The individual detected seropositivity was 1864%. The total percentage of goat flocks exposed to SRLV infection was 51.69%, geographically distributed per region, herd size and production, as reported in Table 1. Seroprevalence was significantly higher in farms of Apulia than in those of Calabria ($P < 0.05$). On the contrary, the herd size did not show significant differences in seroprevalence. The management of the farm, in particular the productive specialization of animals, showed the higher difference in seroprevalence (59.42% vs 43.23% for meat producing farms and milk and meat producing farms, $P < 0.001$, Table 1).

From the bivariate analysis the geographical area and the productive specialization of animals showed to be the main risk factors for SRLV seroprevalence (Table 2). According to recorded results, the herd size cannot be considered a risk factor.

4. Discussion

Early diagnosis of SRLV infection is crucial for monitoring and prevention (Reina et al., 2009) and different diagnostic tools, based on both serological and molecular methods, are available. PCR is sensitive to monitor and point out amino acids changes within target sequences, but as SRLVs are responsible for persistent infections, the antibody screening is the most valuable tool to identify infected animals. As recommended by the World Organization for Animal Health (OIE), ELISA is the most widely used test in the CAEV control programs all over the world (Reina et al., 2009; Mussi et al., 2015), due to its high sensitivity and ability to detect low antibody titers earlier than AGID (Lara et al., 2003).

Though the data on SRLV prevalence observed in different countries cannot be compared each to other because of differences in sensitivity and specificity of the diagnostic tests employed, in sampling methods used and in herd management, bibliography clearly demonstrates that Europe cannot be considered free of SRLV as defined by Office International des Epizooties (Peterhans et al., 2004). In the early 1980s, the pioneer in the control of SRLV was the Netherlands (Houwers et al., 1987), followed by Finland, Germany, Switzerland, France, Spain and Italy that adopted voluntary programs (Péretz et al., 1994; Sihvonen et al., 2000) because, lacking a fit legislation on the matter, the National Official Health Authorities were reluctant to start SRLV control programs. In Italy, however, limited data are currently available on the prevalence of SRLV antibodies in goat population (Pisoni et al., 2006; Gufler et al., 2008), probably due to lack of knowledge on the disease, to poor surveillance from the National Health Authorities and to poor farmers' motivation, with the last being a fundamental aspect of the voluntary control programs (Peterhans et al., 2004). About 11 years ago, a compulsory CAEV eradication program was launched in the Autonomous Province of Bolzano (South Tyrol). It was based on a strict census of small-ruminant population and on the serological testing of all animals, followed by the culling of the seropositive goats. As a consequence of these strict prophylactic measures, during the period 2007–2015, the herd and the individual seroprevalence suffered a drastic reduction (Tavella et al., 2018).

Table 2
Significance of geographical area, herd size and herd specialization as risk factors associated with SRLV virus infection.

	Odds ratio	CI_{95} (%)	Z statistic	P value
Geographic area	1.26	0.98-1.63	1.822	0.0684
Herd size	0.91	0.68-1.21	0.622	0.5341
Production	1.39	1.04-1.89	2.199	0.0279

In the present study, a large-scale survey was carried out in southern Italy to investigate the SRLV status of goats flocks. The investigation demonstrated that SRLV is widespread, with overall seroprevalences of 18.64% and 51.69 at the animal and herd levels, respectively. This finding reasonably reflects the current SRLV herd prevalence in the global Italian goat population, as different provinces with large number of goat flocks were tested, and it is also in accordance with the seroprevalences observed all over the world (Aslantas et al., 2005; Bandeira et al., 2009; Ali et al., 2016; Konishi et al., 2016; Barrero Domínguez et al., 2017; Yang et al., 2017). In particular, our results revealed that both the regions and the production systems are able to affect SRLV seroprevalence, differently from the herd size. Barrero Domínguez et al. (2017), on the contrary, highlighted a key role of herd size factor in CAEV, showing that herd size and management techniques are two associated factors. These difference between Spain and Italy can be the consequence of the different goat producing system adopted in southern Italy, where the mean number of goats per herd is significantly lower than in Spain, and herd management is similar regardless of the dimensional class of each herd. In fact, the management of all herd is based on a semi-intensive rearing system, with pasture associated to indoor housing during nighttime. Herd size influences seroprevalence of SRLV also in Japan. Konishi et al. (2016) observed that large herd sizes (> 10 goats) enrolled a seroprevalence higher than 32% at the herd level, while smaller herd sizes (< 10 goats) registered a lower prevalence (about 10%) at herd level. Probably, this may be due to a different clustering of data set according to herd size, as a consequence of the different situation in southern Italy compared to goat herd management in Japan.

Interestingly, the herd production specialization seems to be the main risk factor in Italy, with higher seroprevalences observed in meat production herds. Herds management is really different in relation to the productive specialization. In the meat producing herds there are neither high-standard hygienic procedures nor animal monitoring as instead occurs in milk producing herds, where animals are managed twice daily for milking and the relationship between dams and kids is well conducted for ensuring an adequate quantitative and qualitative milk yield, in addition to a correct weaning method for kids.

Regardless of the overall prevalence observed, in the presence of a low seropositivity, the objective of the control programs should be to obtain an SRLV-free country, or at most, to maintain the low-prevalence status, whereas countries with a high SRLV prevalence should aim to reduce the virus circulation in small ruminant flocks. Considering that the animal trade represents the main route of SRLV spreading (Shah

et al., 2004; Konishi et al., 2016), animal importation and individual trading among herds should be restricted to herds having the same sanitary status (Tavella et al., 2018). In Italy, no CAEV-free certification is required when importing goats and goat products. Therefore, to reduce and/or minimize the risk of SRLV infection, extensive control and eradication programs should be introduced: i) newborn kids should be removed from their dams right after birth and artificially fed with heat-treated colostrum (56 °C for 1 h), bovine colostrum or commercially available colostrum; ii) infected animals should be segregated from negative goats; iii) animals aged over three months should be periodically tested for SRLV; iv) goats and goat semen should be selected from certified SRLV-free goat farms. Even the implementation of less aggressive and drastic control measures, such as only replacing goats with the offspring of seronegative animals and separating infected from non-infected goats, could significantly reduce virus circulation in infected flocks, thus leading to the progressive control of the infection in highly-infected areas, as observed elsewhere (Péretz et al., 1994). However, since farmers' motivation is a fundamental aspect of the voluntary control programs (Peterhans et al., 2004), the prophylactic measures cannot be implemented by many farmers that do not see immediate economic and productive benefits in controlling the infections (Pérez et al., 2010), mostly because of the greater workload implication and the lack of extra-housing facilities.

It is evident that the seropositivity observed in goat flocks of southern Italy was the direct and obvious consequence of the lack of mandatory SRLV eradication programs. The reduction of the percentage of positivity in the herd and the eradication of the infection could be achieved through different strategies and operating modes that should take into account the structures, the type of breeding and the spread of the infection within the herd.

Our investigation represents only a preliminary study aiming to raise the "SRLV problem", which is greatly underestimated in our country. The next phase in the SRLV surveillance should be the characterization of the genome of the circulating strains and consequently the set-up of specific serological tools that could improve the sensibility of the serological monitoring program.

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Conflict of interest statement

Authors have no competing interests to declare

Declarations of interest

None

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References

Ali, W.H., Saeed, I.K., Mutwakil, S.M., Algezoli, O.A., Mohammed, A.B., Ahmed, I.H., Elhassan, S.M., Ahmed, B.A., Asil, R.M.A., Alsarraj, S.M.A., Abdelgader, A.A., Ali, Y.H., 2016. Determination of antibodies to Caprine arthritis encephalitis virus in goats and sheep in some localities in Sudan. J. Adv. Vet. Anim. Res. 3, 259–262.

Aslantas, O., Özyoruk, F., Pinar, D., Gungor, B., 2005. Serological survey for caprine arthritis encephalitis virus in Damascus and Kilis goats in Hatay, Turkey. Rev. Med. Vet. 156, 402–404.

Bandeira, D.A., de Castro, R.S., Azevedo, E.O., de Souza, Seixas, Melo, L., de Melo, C.B., 2009. Seroprevalence of caprine arthritis-encephalitis virus in goats in the Cariri region, Paraiba state, Brazilian. Vet. J. 180, 399–401.

Barrero Domínguez, B., Luque, I., Maldonado, A., Huerta, B., Sánchez, M., Laguna, J.G., Astorga, R., 2017. Seroprevalence and risk factors of exposure to caprine arthritis-encephalitis virus in southern Spain. Vet. Rec. 180, 226.

Bertolotti, L., Reina, R., Mazzei, M., Preziuso, S., Camero, M., Carrozza, M.L., Cavalli, A., Juganaru, M., Profiti, M., De Meneghi, D., Perona, G., Renzoni, G., Tursi, M., Bertoni, G., Rosati, S., 2013. Small ruminant lentivirus genotype B and E interaction: evidences on the role of Roccaverano strain on reducing proviral load of the challenging CAEV strain. Vet. Microbiol. 163, 33–41.

Gjerset, B., Storset, A.K., Rimstad, E., 2006. Genetic diversity of small-ruminant lentiviruses: characterization of Norwegian isolates of caprine arthritis encephalitis virus. J. Gen. Virol. 87, 573–580.

Glaria, I., Reina, R., Crespo, H., de Andrés, X., Ramírez, H., Biescas, E., Pérez, M.M., Badiola, J., Luján, L., Amorena, B., de Andrés, D., 2009. Phylogenetic analysis of SRLV sequences from an arthritic sheep outbreak demonstrates the introduction of CAEV-like viruses among Spanish sheep. Vet. Microbiol. 138, 156–162.

Grego, E., Lacerenza, D., Arias, R.R., Profiti, M., Rosati, S., 2009. Serological characterization of the new genotype E of small ruminant lentivirus in Roccaverano goat flocks. Vet. Res. Commun. 33, 137–140.

Gufler, H., Moroni, P., Casu, S., Pisoni, G., 2008. Seroprevalence, clinical incidence, and molecular and epidemiological characterization of small ruminant lentivirus in the indigenous Passirian goat in northern Italy. Arch. Virol. 153, 1581–1585.

Houwers, D.J., König, C.D.W., Bakker, J., de Boer, M.J., Pekelder, J.J., Sol, J., Vellema, P., de Vries, G., 1987. Maedi-visna control in sheep. III. Results and evaluation of a voluntary control program in the Netherlands over a period of four years. Vet. Q. 9, 29–36.

Konishi, M., Hayama, Y., Shirafuji, H., Kameyama, K., Murakami, K., Tsutsui, T., Akashi, H., 2016. Serological survey of caprine arthritis-encephalitis virus infection in Japan. J. Vet. Med. Sci. 78, 447–450.

Lara, M.C.C.S.H., Birgel Junior, E.H., Fernandes, M.A., Birgel, E.H., 2003. Infecção experimental do vírus da artrite encefalite dos caprine semcabritos. Arq. Inst. Biol. (Sao Paulo) 70, 51–54.

Martínez-Navalón, B., Peris, C., Gómez, E.A., Peris, B., Roche, M.L., Caballero, C., Goyena, E., Berriatua, E., 2013. Quantitative estimation of the impact of caprine arthritis encephalitis virus infection on milk production by dairy goats. Vet. J. 197, 311–317.

Minguijón, E., Reina, R., Pérez, M., Polledo, L., Villoria, M., Ramírez, H., Leginagoikoa, I., Badiola, J.J., García-Marín, J.F., de Andrés, D., Luján, L., Amorena, B., Juste, R.A., 2015. Small ruminant lentivirus infections and diseases. Vet. Microbiol. 181, 75–89.

Mussi, J.M.S., Gouveia, A.M.G., Cortez, A., Lage, A.P., de Sá Guimarães, A., Heinemann, M.B., 2015. Use of serological diagnostic techniques in the control and eradication of caprine arthritis encephalitis: an update. Braz. J. Vet. Res. Anim. Sci. 52, 283–297.

Olech, M., Rachid, A., Croisé, B., Kuźmak, J., Valas, S., 2012. Genetic and antigenic characterization of small ruminant lentiviruses circulating in Poland. Virus Res. 163, 528–536.

Péretz, G., Bugnard, F., Calavas, D., 1994. Study of a prevention program for caprine arthritis encephalitis. Vet. Res. 25, 322–326.

Pérez, M., Biescas, E., de Andres, X., Leginagoikoa, I., Salazar, E., Berriatua, E., Reina, R., Bolea, R., de Andres, D., Juste, R., Cancer, J., Gracia, J., Amorena, B., Badiola, J., Lujan, L., 2010. Visna/maedi virus serology in sheep survey, risk factors and implementation of a successful control programme in Aragon (Spain). Vet. J. 186, 221–225.

Peterhans, E., Greenland, T., Badiola, J., Harkiss, G., Bertoni, G., Amorena, B., Eliaszewicz, M., Juste, R., Krassnig, R., Lafont, J., Lenihan, P., Pétursson, G., Pritchard, G., Thorley, J., Vitu, C., Mornex, J., Pépin, M., 2004. Routes of transmission and consequences of small ruminant lentiviruses (SRLVs) infection and eradication schemes. Vet. Res. 35, 257–274.

Pisoni, G., Bertoni, G., Boettcher, P., Ponti, W., Moroni, P., 2006. Phylogenetic analysis of the gag region encoding the matrix protein of small ruminant lentiviruses: comparative analysis and molecular epidemiological applications. Virus Res. 116, 159–167.

Reina, R., Berriatua, E., Luján, L., Juste, R., Sánchez, A., de Andrés, D., Amorena, B., 2009. Prevention strategies against small ruminant lentiviruses: an update. Vet. J. 182, 31–37.

Reina, R., Bertolotti, L., Dei Giudici, S., Puggioni, G., Ponti, N., Profiti, M., Patta, C., Rosati, S., 2010. Small ruminant lentivirus genotype E is widespread in Sarda goat. Vet. Microbiol. 144, 24–31.

Shah, C., Böni, J., Huder, J.B., Vogt, H.R., Mühlherr, J., Zanoni, R., Miserez, R., Lutz, H., Schüpbach, J., 2004. Virology 319, 12–26.

Sihvonen, L., Nuotio, L., Rikula, U., Hirvelä-Koski, V., Kokkonen, U.M., 2000. Preventing the spread of maedi-visna in sheep through a voluntary programme in Finland. Prev. Vet. Med. 47, 213–220.

Tavella, A., Bettini, A., Ceol, M., Zambotto, P., Stifter, E., Kusstatscher, N., Lombardi, R., Nardeli, S., Beato, M.S., Capello, K., Bertoni, G., 2018. Achievements of an eradication programme against caprine arthritis encephalitis virus in South Tyrol, Italy. Vet. Rec. 182, 51.

Thrusfield, M., 2004. Epidemiologia Veterina´ria, second ed. Roca Press, Sao Paulo.

Yang, W.-C., Chen, H.-Y., Wang, C.-Y., Pan, H.-Y., Wu, C.-W., Hsu, Y.-H., Su, J.-C., Chan, K.-W., 2017. High prevalence of caprine arthritis encephalitis virus (CAEV) in Taiwan revealed by large-scale serological survey. J. Vet. Med. Sci. 79, 273–276.