



Achievements of an eradication programme against caprine arthritis encephalitis virus in South Tyrol, Italy

Alexander Tavella,¹ Astrid Bettini,¹ Marco Ceol,¹ Paolo Zambotto,² Ernst Stifter,² Natashia Kusstatscher,¹ Rosalba Lombardi,² Stefano Nardeli,³ Maria Serena Beato,³ Katia Capello,⁴ Giuseppe Bertoni⁵

Small ruminant lentivirus infections in goats affect both production and animal welfare. This represents a threat to the qualitative and quantitative growth of goat farming, recently observed in mountainous regions such as the Autonomous Province of Bolzano – South Tyrol (Italy). To monitor and eradicate the caprine arthritis encephalitis virus in this goat population, a compulsory eradication campaign was launched, based on a strict census of small ruminants and yearly serological testing of all animals, followed by the consequent culling of seropositive individuals. The campaign succeeded in completely eliminating cases of clinical disease in goats, while drastically reducing the seroprevalence at the herd as well as individual animal level. The serological outcome of the introduced control measures was determined using commercially available ELISA kits, demonstrating their suitability for use in this type of campaign, aimed at reducing seroprevalence as well as clinical manifestations of these infections. However, this clear success is diminished by the failure to achieve a complete eradication of these viruses. The reasons leading to the observed tailing phenomenon and the occurrence of new infections in already sanitised flocks are discussed and implementation of further measures are proposed.

Introduction

Caprine arthritis encephalitis virus (CAEV) and Maedi visna virus (MVV) were considered as species-specific pathogens of goats and sheep, respectively. Nowadays they are referred to as small ruminant lentiviruses (SRLV) and are considered to represent a genetic continuum, whose principal representatives, that is, SRLV A (MVV), B (CAEV) and C (a particular genotype detected in Norway), were shown to cross the species barrier between goats and sheep.^{1–5} Five SRLV genotypes (A–E) have been described so far, with A, B and E further subdivided in subtypes A1–A15, B1–B3 and E1–E2.^{4,6} As an exception, genotype E appears to be goat-specific.^{7,8} Noteworthy are the findings of several

authors reporting genomic sequences from sheep with higher identities to CAEV than to MVV,^{9–11} indicating a possible common ancestral genotype. These viruses are widespread worldwide with the exception of New Zealand and Australia, which are considered free of SRLV A, and Iceland, which eradicated SRLV after a devastating epizootic outbreak originating from the import of SRLV-infected karakul sheep in the early 1930s.^{12–20} Genotype B, and in particular the B1 subtype, is considered the most virulent genotype for goats, whereas in sheep genotype A is the principal cause of clinical manifestations.^{2,6}

SRLVs are known to be the cause of a chronic multi-systemic inflammatory disease characterised by a long incubation period and a lifelong persistent infection.²¹ Infections due to SRLV are associated with a progressive degenerative disease that involves, at different stages, the joints and mammary glands, causing progressive chronic arthritis and non-suppurative indurative mastitis, along with other pathological manifestations, affecting both the respiratory system and CNS.⁶ Clinical signs are apparent in less than 30 per cent of infected goats and sheep, pointing to the importance of host genetic factors. Nonetheless, all seropositive animals represent a potential source of infection.^{22–26}

The main routes of viral transmission are ingestion of colostrum and milk from infected animals and horizontal transmission via respiratory secretions between

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¹Laboratory for Serology and Technical Assistance, Istituto Zooprofilattico Sperimentale delle Venezie, Bolzano, Italy

²Veterinary Service, Servizio Veterinario Provinciale, Bolzano, Italy

³Diagnostic Virology Laboratory, Istituto Zooprofilattico Sperimentale delle Venezie, Legnaro, Italy

⁴Direzione Sanitaria, Istituto Zooprofilattico Sperimentale delle Venezie, Legnaro, Italy

⁵Vetsuisse Faculty, Institute of Virology and Immunology, University of Bern, Bern, Switzerland

E-mail for correspondence: atavella@izsvenezie.it

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individuals.^{15 27–29} Horizontal transmission, however, is considered more important in sheep than goats.^{30–32} Transmission to human beings is not considered a potential threat because of the absence of functional receptors at the surface of human cells³³; further studies should focus on the possibilities that recombination and mutation of the virus could lead to the generation of a potentially zoonotic virus.

Caprine arthritis encephalitis has a negative impact on several performance parameters, leading to economic losses.^{34–36} Clinical signs in SRLV-infected animals include a decrease in milk productivity and milk quality, loss of weight, and premature death; furthermore, animals at advanced stages of the disease present a significantly reduced bodyweight at slaughter, and therefore may not be suitable for consumption.^{15 37 38} Along with poor animal welfare and environmental protection,¹⁵ these economic factors have contributed to the growing demand for control and eradication programmes to be undertaken by countries around the world.^{6 39 40} The most commonly used prevention and eradication strategies are the testing and removal of serologically positive animals, often combined with the immediate separation of lambs and kids of seropositive mothers at birth for rearing on CAEV-negative or bovine colostrum, as well as the separation of seropositive from seronegative flocks and the strict control of live animal trade.^{15 41 42} The success of such programmes depends on the reliability of the adopted diagnostic procedures, primarily based on the detection of specific antibodies in infected animals. Seroconversions can be detected a few weeks after infection, although some animals only seroconvert several months after infection. Infected animals, transiently or permanently seronegative, are also known to occur.^{43–45}

Serology is the most convenient technique to diagnose SRLV infections, even though other laboratory methods for the detection of antibodies or the virus genome sequences are available.^{39 40 43 44 46}

The Autonomous Province of Bolzano – South Tyrol (Italy) is the northernmost province of Italy and is located on the border with Switzerland and Austria. It is geographically and morphologically marked by a pronounced subalpine to alpine terrain. The characteristics of the territory determine the conformation and size of the typical mountain farms, with an average of 11 goats per herd. South Tyrol's animal husbandry is characterised by a multispecies farming system, where goats, sheep and cattle can be found in the same barn. In 2015, the total number of goats in the Province of Bolzano amount to 23 806 animals held on 2207 farms; 30 per cent of these are mixed goat–sheep breeding system farms. The increasing number of goats is based on the recent growing demand for caprine dairy products, both by breeders and consumers. On the other hand, the ovine population consists of 42 187 animals. From the economical as well as emotional point of view, goat

breeding plays an important role in the agricultural landscape in the Province of Bolzano. The adverse impact of SRLV infections on goat milk and cheese production, as well as the negative effect of these infections on the successful breeding of the Passirian goat, an endangered local breed, has driven the local authorities to implement an eradication programme targeting CAEV in goats. Preliminary serological surveys and a pilot project launched between December 2003 and May 2004 showed that the preconditions for a successful eradication campaign, that is, a relatively low seroprevalence and the motivation of the participating goat breeders, were fulfilled.^{28 47 48} The compulsory CAEV eradication programme was therefore started in November 2007. In this publication, we describe the results obtained so far and discuss the problems encountered during the implementation phase and their impact on the future of this eradication plan.

Materials and methods

Eradication programme

The compulsory CAEV eradication programme, which started in November 2007 and is still ongoing, is subdivided in yearly recurrent serological testing campaigns lasting from November 1 to April 30 of the following year, with the culling of all infected animals. Based on the pilot study conducted in 2003–2004, all goat herds within the territory of the Autonomous Province of Bolzano (on average 2086 farms/serological testing campaign) were included in the eradication programme. During each campaign, both female and male goats older than six months of age (on average 19,487 animals/campaign) were tested for anti-CAEV antibodies. All goats that reacted positively or inconclusively to the analysis were considered CAEV-infected. Furthermore, non-tested kids (younger than six months of age) born from seropositive does, which had been fed colostrum from the infected mother and/or had been in close contact with her, were considered infected as well. All herds with at least one infected animal were considered infected. In all multispecies farming systems with at least one positive goat, all sheep were analysed for anti-CAEV antibodies as well, and seropositive sheep were culled. Furthermore, whenever more than 30 per cent of a herd had tested positive, all animals were eliminated. The precautionary actions, imposed by the decree of the veterinary authorities (Decree of the Veterinary Service n. 351624⁴⁹), were as follows: all positive goats were subjected to precautionary confiscation (they were forbidden from auctions, market-places and grazing lands), had to be kept separated from all other animals of the herd and had to be culled within 60 days from the laboratory result.

Herds and animals

All goat herds within the territory of the Autonomous Province of Bolzano (on average 2086 farms/serological testing campaign) were included in the eradication

programme. The characteristics of each herd are recorded in the National and Provincial Small Ruminant Database; identification of small ruminants in the Autonomous Province of Bolzano is implemented in accordance with regulation 21/2004/CE. Since 2013, the identification of goats has been carried out exclusively by the use of electronic devices, such as ruminal bolus and electronic ear tags.

Serological investigation

Blood samples were drawn from the jugular vein using vacuum blood collection tubes with a clotting activator manufactured by Vacutest Kima (Arzergrande, Italy) and centrifuged for 3 minutes at 1646 g to obtain the serum. Serological analyses were performed by the serological laboratory of the Institute for Animal Disease Control—Istituto Zooprofilattico Sperimentale delle Venezie—in Bolzano. Serum samples were tested from November 1, 2007 to April 30, 2011 using a test kit from Pourquier (ELISA Maedi-Visna/CAEV Serum Verification, V.P032/01, Institute Pourquier, Montpellier, France) with a reported specificity (Sp) \geq 99.8 per cent and a sensitivity (Se)=97.9 per cent, and from November 1 2012 onwards, with an antibody test kit from IDEXX (MVV/CAEV p28 Ab Screening Test, IDEXX, France) (Sp \geq 99.8 per cent and Se =97.9 per cent). Both ELISA kits use the p28 recombinant viral capsid protein as the antigen. Tests were performed according to manufacturers' instructions. Each year, the seroprevalence was calculated using a 95 per cent CI. To evaluate the significance of trend in seroprevalence across different years, the Cochran-Armitage test was applied.

Results

From November 1, 2007 to April 30, 2015, a total number of 155 894 goat blood samples were analysed for SRLV antibody. The seroprevalence decreased from 13.9 per cent (95 per cent CI 13.4 to 14.4 per cent) in 2007–2008 to 0.3 per cent (95 per cent CI 0.2 to 0.3 per cent) in 2014–2015 (Table 1). This trend in seroprevalence is statistically significant (P <0.001). A similar trend could also be determined for the herd prevalence. The observed herd prevalence in 2007–2008 was 32 per cent

TABLE 1: Seroprevalence of small ruminant lentiviruses infections in goats subdivided in prevention campaigns

Serological testing campaigns	Tested goats	Positive goats	Seroprevalence (%)	95% CI
2007–2008	18,475	2576	13.9	13.4 to 14.4
2008–2009	17,016	657	3.9	3.6 to 4.2
2009–2010	18,163	329	1.8	1.6 to 2.0
2010–2011	19,078	196	1.0	0.9 to 1.2
2011–2012	19,665	134	0.7	0.6 to 0.8
2012–2013	19,315	96	0.5	0.4 to 0.6
2013–2014	22,756	105	0.5	0.4 to 0.6
2014–2015	21,417	65	0.3	0.2 to 0.4

There is a statistically significant trend in the progress of seroprevalence found in the 8-year period (P <0.001).

TABLE 2: Prevalence of positive goats herds in the Autonomous Province of Bolzano subdivided in prevention campaigns

Serological testing campaigns	Tested herds	Positive herds	Prevalence (%)	95% CI
2007–2008	1927	617	32.0	29.9 to 34.1
2008–2009	1975	443	22.4	20.6 to 24.3
2009–2010	2001	170	8.5	7.31 to 9.8
2010–2011	2086	119	5.7	4.7 to 6.7
2011–2012	2325	88	3.8	3.0 to 4.6
2012–2013	2039	56	2.7	2.1 to 3.6
2013–2014	2207	39	1.8	1.6 to 2.0
2014–2015	2128	32	1.5	1.3 to 1.7

There is a statistically significant trend in the progress of prevalence (P <0.001).

(95 per cent CI 29.9 to 34.1 per cent) and decreased over the coming years to 1.5 per cent (95 per cent CI 1.3 to 1.7 per cent) in 2014–2015 (Table 2).

Discussion

The drastic reduction in herd and individual seroprevalence during the period 2007–2015 indicates that the implemented eradication programme was highly successful. The statistical trend in prevalence was highly favourable during the first years of the eradication programme (from 13.9 to 1.0 per cent in the first four serological testing campaigns). In contrast, in the last years of the programme, we observed a tailing phenomenon (from 0.7 to 0.3 per cent in the second four serological testing campaigns) with the occurrence of new positive cases (Table 1).

This phenomenon is inherent to SRLV eradication programmes and relates to the complex biology of these viruses.^{2 50} Genetic diversity, along with an absence of universal protocols able to detect all genotypes and subtypes of the virus, represents an important issue in testing and diagnosing SRLV-infected animals.^{39 44 51} The ELISA used in this work has a high Sp and Se . The Office International Des Epizooties World Organisation for Animal Health provides information on the validity of ELISA with respect to other assays: it states that several indirect ELISA protocols have shown higher Sp and Se against a standard comparison (Western blot or radioimmunoprecipitation).^{52–54} Before the establishment of the eradication programme, the high prevalence of CAEV in South Tyrol and the infection risks were partially related to the transhumance practices occurring in the summertime, during which time sheep and goats from different herds with unknown CAEV sanitary status were moved to grazing areas situated in the mountains. With the implementation of the programme, several control practices were introduced, as suggested in the final report of the first consensus conference on SRLV.¹⁵ As required by the eradication plan, nowadays only herds with the same sanitary status are allowed to be grazed on common alpine pastures. The trade of live animals is controlled by the veterinary authorities, even though occasional illegal trading has occurred, leading to the reinfection of these herds. Cross-species

transmission between sheep and goats may be particularly relevant in the context of the multispecies farming^{55–57}; in South Tyrol, we are actively monitoring such occurrences. In the context of analysing the occurrence of few remaining seropositive animals in our province, it is important to emphasise the fact that the Autonomous Province of Bolzano implemented a programme focused exclusively on goats. Except for promiscuous herds, sheep were not included in the eradication effort and may well function as SRLV reservoir. We are planning different studies to assess the risk of the typical multispecies breeding system in our region, with the goal of adapting the eradication programme. Furthermore, the presence of wild ruminants in our grazing areas could impact a herd's sanitary status, as transmission of SRLV between goats, sheep and wild ruminants has been described.^{5 58–60}

In spite of the fact that trading individuals between herds and animal importation is strictly limited to herds with the same sanitary status, we identified the trade of animals as one of the two principal causes of reinfection. The second most crucial factor was the potential presence of false-negative animals in the sanitised herds, resulting from the use of a single serological assay (A. Tavella, unpublished observations).⁵¹ It is common for farmers in South Tyrol to use the same buck in several herds; even though this should only occur between farms with the same sanitary status, unfortunately it sometimes occurs between seropositive and seronegative herds. The use of the same breeding buck in herds with different sanitary status may be an important risk factor undermining the eradication effort.^{56 61}

Finally, the goal of this mandatory programme was to determine and reduce the seroprevalence of CAEV in the goat population of South Tyrol, thereby eliminating the CAEV-induced clinical manifestations and potentially eradicating the virus. The first two aims were successfully achieved and CAEV-induced clinical disease in goats is no longer observed in the Autonomous Province of Bolzano – South Tyrol. This demonstrates that the use of commercially available ELISA kits is suitable and sufficient for the assessment of CAEV control programmes aimed at reducing seroprevalence as well as clinical manifestations of this virus. However, the emergence of new infectious cases or of singleton reactors in the last years of the eradication programme indicates that the complete eradication of SRLV from a population cannot be easily achieved. In order to overcome this problem, the study and development of additional diagnostic tools are required.

As a consequence of these findings, we are planning a second phase in the eradication programme. We will characterise the genomic and antigenic characteristics of the circulating SRLV involved in reinfections, consequently adapting the serological tools used in the programme. The absence of clinical arthritis in our goats, strongly suggests that, in analogy to the Swiss situation,

the virulent genotype B SRLV was almost completely eradicated in South Tyrol.⁵⁰ The monitoring of this particular genotype will be increased by applying highly sensitive tests and by isolating and molecularly characterising viruses from seropositive animals selectively slaughtered for this purpose.

In view of the important financial and human resource investment in such a compulsory eradication programme, it is extremely important to increase the efforts in this final difficult phase of the programme to prevent the reinfection of goat flocks and the spread of SRLV infections within our territory.

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Competing interests None declared.

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Achievements of an eradication programme against caprine arthritis encephalitis virus in South Tyrol, Italy

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