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Dear Editor

I would like to submit the manuscript entitled “Small ruminant lentivirus genotype E and B interaction: evidences on the role of Roccaverano strain (Genotype E) on reducing proviral load of the challenging CAEV strain (Genotype B)” for publication on Vaccine. It describes a double infection in goats using a field strain of small ruminant lentivirus naturally deleted for dUTPase and VPR-like gene (Roccaverano strain) and antigenically distinct from Caprine Arthritis Encephalitis Virus (CAEV), which was used as challenge strain. Results demonstrate that proviral load of CAEV in double infected animals is statistically lower than in single infected group and suggest the potential application of Roccaverano strain as live attenuated vaccine for control of SRLV in goat population, heavily infected with pathogenic CAEV strains. We believe that this study would be of interest not only for scientists involved in small ruminant lentivirus research. Thank you in advance for your consideration.

The corresponding author

Highlights

- Goats were infected or mock infected with low pathogenic SRLV genotype E
- 20 weeks later both groups were infected with CAEV-Cork
- Proviral load of CAEV was reduced in double infected animals
- CAEV was not detected in kids raised by double infected animals

Abstract

Live attenuated vaccines provide the most consistent protective immunity in experimental models of lentivirus infections. In this study we tested the hypothesis that animals infected with a naturally attenuated small ruminant lentivirus field strain of genotype E may control a challenge infection with a virulent strain of the caprine arthritis encephalitis virus (CAEV-CO). Within genotype E, Roccaverano strain has been described as low pathogenic since decreased arthritic pathological indexes were recorded in Roccaverano-infected animals compared to animals of the same breed infected with genotype B strains. Moreover, under natural conditions, animals double-infected with genotype B and E appear less prone to develop SRLV-related disease, leading to a putative protective role of Roccaverano strain. Here we present evidence that goats experimentally infected with the avirulent genotype E SRLV-Roccaverano strain control the proviral load of a pathogenic challenge virus (CAEV-CO strain) more efficiently than naïve animals and appear to limit the spread of histological lesions to the contralateral joints.

Small ruminant lentivirus genotype E and B interaction: evidences on the role of Roccaverano strain (Genotype E) on reducing proviral load of the challenging CAEV strain (Genotype B)

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Abstract

Live attenuated vaccines provide the most consistent protective immunity in experimental models of lentivirus infections. In this study we tested the hypothesis that animals infected with a naturally attenuated small ruminant lentivirus field strain of genotype E may control a challenge infection with a virulent strain of the caprine arthritis encephalitis virus (CAEV-CO). Within genotype E, Roccaverano strain has been described as low pathogenic since decreased arthritic pathological indexes were recorded in Roccaverano-infected animals compared to animals of the same breed infected with genotype B strains. Moreover, under natural conditions, animals double-infected with genotype B and E appear less prone to develop SRLV-related disease, leading to a putative protective role of Roccaverano strain. Here we present evidence that goats experimentally infected

with the avirulent genotype E SRLV-Roccaverano strain control the proviral load of a pathogenic challenge virus (CAEV-CO strain) more efficiently than naïve animals and appear to limit the spread of histological lesions to the contralateral joints.

Keywords

Small ruminant lentivirus, genotype E, Roccaverano, experimental infection, immunization strategy

Introduction

1 Small ruminant lentiviruses (SRLV) are distributed around the world causing a multisystemic
2 disease in sheep and goats leading to production losses as well as to consequences in animal trade
3 and welfare. Target organs typically, the lungs, udder, carpal joints and the central nervous system,
4 are affected by inflammatory process characterized by a dense cellular infiltrate of mononuclear
5 leukocytes, mainly lymphocytes and, to a lesser extent, B cells, plasmatic cells and macrophages
6 the latter being the SRLV target cell in vivo [1-3]. Control is mainly based on serological diagnosis
7 and culling of the seropositive animals. However, detection of antibodies is affected by antigenic
8 heterogeneity among SRLV genotypes, which makes serological testing an inaccurate control
9 method if heterologous diagnostic antigen is employed [4, 5].

10 SRLV are genetically divided into five genotypes [6-8]. Among them, it is well known the
11 association between the genotype B and the caprine arthritis encephalitis disease, causing a threat
12 on animal welfare and in milk production of small ruminants. In contrast, the lastly described
13 genotype E, was first isolated in few local Roccaverano breed goats in Piedmont, Italy, where no
14 SRLV related clinical signs were reported by breeders or practitioners. Histopathological score was
15 also reduced, when evaluated under natural and experimental conditions (Grego, unpublished
16 observations). These findings together with natural deletions of the dUTPase subunit of the pol gene
17 and the vpr-like gene, within viral genome, led us to tentatively term Roccaverano strain, as low
18 pathogenic caprine lentivirus [9, 10]. In addition, the Roccaverano strain was identified in a flock in
19 which animals were found co-infected with the pathogenic CAEV-like genotype B, with no
20 apparent development of SRLV-related disease. Current opinion among local breeders and
21 practitioners is the breed resistance of Roccaverano goat to CAEV infection, compared to Alpine
22 and Saanen breeds. However, Roccaverano breed goats infected by field genotype B strains did
23 develop arthritis [5]. In the light of the discovery of genotype E infection, preexisting to the
24 introduction of CAEV-like strains in this local population, a new approach should be taken into

account to explain breed resistance: could genotype E infection act as live attenuated vaccine, able to induce resistance to superinfection versus heterologous strains?

We recently evaluated the immunological parameters in goats experimentally infected with Roccaverano strain using homologous and heterologous antigens. Results clearly indicated that humoral and T-cell proliferation responses were strictly detected against recombinant antigens from the homologous genotype, while cytotoxic-T-lymphocyte (CTL) activity was not strain specific, being surprisingly higher against genotype B infected antigen presenting cells [10]. Thus CTL activity is likely the sole adaptive immune response which could be associated to protection against heterologous strain. Following these experiments, in this study we have explored the potential protective role of genotype E in vivo under strictly controlled conditions by infecting goats with Roccaverano strain followed by a challenge with pathogenic CAEV-CO strain. Results indicate that previous infection with genotype E results in a decreased proviral load of the challenge strain, acting itself as a potential natural prevention strategy to control SRLV infection.

Materials and Methods

Cells and Viruses

Caprine peripheral blood mononuclear cells (PBMC) were obtained from SRLV-free animals and derived macrophages (BDM) were allowed to differentiate for 9-15 days as described [9]. BDMs were used to propagate and titrate Roccaverano strain. Goat synovial membrane primary cell line was used to propagate and titrate CAEV Cork strain.

Low pathogenic SRLV strain Roccaverano (genotype E, subtype E1) was isolated and characterized in previous studies [11]. The pUC9kb-CAEV [12] and pCAEVLTR-cat [13] plasmids containing two fragments that allow reconstitution of replication-competent CAEV-CO virus upon co-transfection of primary goat synovial membrane cells (GSM) were kindly supplied by Dr. R. Vigne (Marseille, France).

Animals

1 Animals from a certified SRLV-free herd formed by Roccaverano breed goats as previously
2 described [5], were used in this study. A total of 22 female goats were purchased as weaned kids
3 and introduced into the experimental facilities at the Faculty of Veterinary Medicine, University of
4 Turin, Grugliasco, Italy (CISRA FMV UNITO) 18 months before the experimental infection.
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10 Animals were tested monthly and found consistently negative for SRLV antibodies using genotype
11 A, B and E-derived antigens for a one-year period. Animals included in experimental and control
12 groups had similar age distribution, ranging from 9 to 24 months. During the experiment, a total of
13 22 goat kids born between 243 and 355 days post challenge. In detail, 7 kids born from animals
14 infected with Roccaverano strain, 11 from animals infected with CAEV-CO and 4 from uninfected
15 animals.
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18 Experiments were carried out in compliance with the relevant National legislation on experimental
19 animals and animal welfare, upon authorization by the competent authority (Italian Ministry of
20 Health-Directorate General Animal Health-Office VI; permit no. 07/2009B).
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Experimental design

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Animals were divided into three groups. Group A included 8 animals that were subjected to experimental infection with Roccaverano strain inoculated intra-tracheally with 2 ml of 2.5×10^5 TCID₅₀/ml. After 4 months, animals were challenged with CAEV-CO through direct inoculation of 0.5 ml of 10^6 TCID₅₀/ml per animal in the right carpal joint, after removal of an equal volume of synovial fluid. Group B included 8 animals infected with the CAEV-CO strain at the same time, dose and challenge method as group A. Group C included 6 healthy animals, as control group.

EDTA blood samples were obtained in all groups 15 days prior to infection (-15), at weekly interval until day 49, and bi-weekly until day 132, corresponding to the day of the challenge. After the CAEV-CO infection, EDTA blood samples were collected from all animals at weekly interval until

1 day 169, bi-weekly until day 205, and then every 4-6 weeks until the end of experiment which
2 lasted over one year after challenge with a total of 28 time points. From each blood sample plasma
3 was collected after centrifugation and stored at -20°C for serological analysis and buffy coats were
4 recovered, resuspended in PBS and stored at -20°C until DNA extraction for PCR studies.
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8 After parturition, colostrum was sampled and analyzed for proviral load as well as antibodies and
9 blood samples were collected from all kids bi-weekly until the end of the experiment.
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11 12 13 14 15 *Serological analysis*

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18 In order to evaluate the serological response against each infecting strain, a genotype-specific and a
19 strain specific ELISA tests were applied. The first employs a recombinant P16-25 fusion protein
20 derived from genotype B and E, which has been proven in previous study to discriminate between
21 SRLV genotype B or E infection [5]. The second ELISA test is based on strain specific SU5
22 synthetic peptides which have been successfully described as specific and precocious (early) marker
23 of infection [14, 15]. Synthetic peptide sequences of Roccaverano and CAEV-CO strains used in
24 the second ELISA test (strain specific) were QVRAYTYGVIEMPTGYETPTIRRR and
25 KVRAYTYGVIEMPENYAKTRIINRK respectively. All animals were tested at each time point
26 and seroconversion against the two variants of the same antigen was independently monitored.
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42 *DNA extraction and proviral load quantification*

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44 DNA was isolated from buffy coats with DNeasy Blood and Tissue kit (Qiagen, Germany) and
45 quantified by fluorimetry with Picogreen dsDNA Quantitation kit, (Invitrogen, Carlsbad, CA). Two
46 sets of strain-specific primers and probes were designed with Beacon Designer 7 (Premier Biosoft
47 International) to set up a duplex q-PCR targeting pol sequences. Primer and probe sequences are
48 reported in Table 1. PCR reactions were carried out in triplicate with Quantitech Multiplex PCR kit
49 (Qiagen) in a volume of 25 µl with 200nM each primer, 300 nM probe and 5 µl of DNA. After an
50 initial activation at 95°C for 15 min, reactions were subjected to 50 cycles of 94°C for 15 sec and
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60°C for 1 min. Negative controls were included in each assay, as well as serially diluted positive controls allowing quantification through estimations on a standard curve. In more details, two different plasmids carrying Roccaverano and CAEV-CO pol gene fragments spanning the real-time amplicons were prepared. The former included a 3,309 bp insert (nucleotides 3782 to 7090 along Roccaverano genome) while the latter carried a 453 bp fragment, corresponding to nucleotides 1589 to 2131 in CAEV-CO genome. Serial dilutions from 10^6 to 5 copies/reaction) of both plasmids were included in each assay to generate standard curves.

The coefficient of variation (COV) of the proviral copy number per reaction of the three replicas for each DNA template was calculated. A threshold value of 0.28 was established for DNAs having more than 10 copies per reaction and outliers were excluded from further analysis. No COV analysis was applied to DNA samples carrying less than 10 copies per reaction. Proviral load values were expressed as the average copy number per microgram of template DNA.

T cell proliferation

Measurement of T cell proliferation against homologous and heterologous antigens was carried out as described elsewhere [10]. Briefly, PBMCs prepared by buffy coat centrifugation on Ficoll gradient were plated in 96-well plates at a concentration of 10^5 cells/well in RPMI-1640 medium (Sigma–Aldrich Company Ltd) supplemented with 2mM L-glutamine, 50 μ M beta-mercaptoethanol, 100 U penicillin and 100 μ g streptomycin/ml, 10% FBS (RPMI 10). PBMCs were incubated in quadruplicate with recombinant heterologous (genotype B) or autologous (genotype E) P25 antigen, or GST (as negative control) at equimolar amounts. Antigens were plated at 25, 12 and 6 μ g/ml in 200 μ l and after a five-day incubation, cells were labelled with 1 μ Ci of [3 H] thymidine (Amersham) for 5 h. Incorporated radioactivity was determined using a Filter Cell Harvester 1540 (Wallac) and a Beta counter. Proliferation was measured as a stimulation index (SI) normalizing incorporated radioactivity in P25 wells with that obtained in the GST wells. The SI was calculated for each antigen using the formula $SI = \text{cpm with antigen} / \text{cpm with GST protein}$.

1 An individual animal was considered to show positive T cell reactivity if the SI was greater than 3
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6 in at least two antigen dilutions.

7 *Clinical and histopathological examination*

8 In order to determine the development of clinical arthritis, the circumference ratio between carpus
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10 and the contralateral metacarpus (c/m) was monitored. As commonly known, ratios above 1.8
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12 indicated the presence of clinical arthritis [16].

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15 Following euthanasia of experimental goats, samples from the left and the right carpal joints were
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17 collected and fixed in buffered formalin for 48 hours. Two synovial tissue samples from each
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19 sample were embedded in paraffin for histopathology. Four-micron sections were stained with
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21 haematoxylin and eosin staining (H&E) according to routine procedures. The slides were observed
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23 blindly by two independent pathologists monthly for three times. Each time, five pictures at 20X
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25 were evaluated from each sample. On the basis of the enlargement of the synovial lining cell layer,
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27 the density of the resident cells and the inflammatory infiltrate, histological lesions were blindly
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29 recorded as no lesion, mild, moderate or severe lesions. The mean value of the three lesion scores
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31 was considered as the final score. Final lesion score of the left and the right synovial samples were
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33 compared.

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44 Real time PCR sensitivity and specificity were analyzed with Rotor-Gene software version 1.7
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46 (Qiagen). Blood proviral load, clinical and histopathological lesion scores from groups A and B
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48 were analyzed and compared using Wilcoxon rank-sum test at each time point, considering $\alpha=0.05$.
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50 Fisher's exact test was applied for T cell proliferation frequencies of positive animals. All statistical
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52 tests were performed using R statistical software [17].
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59 **Results**

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Serological response

1 Seroconversion against Roccaverano and CAEV-CO strains was evaluated using indirect ELISA
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3 containing p16-25 recombinant protein and SU5 synthetic peptide derived from each of the two
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5 genotypes (Fig 1).
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8 In animals belonging to the group A, serological response was mounted exclusively against
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10 homologous p16-25 antigen within 14 and 104 days. Peaks were reached at 44 and 72 days p.i. and
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12 afterwards decreased transiently to become consistently positive upon 141 days drawing the typical
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14 two-phase curve. After CAEV-CO challenge, group A developed antibodies against genotype B
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16 antigen, thus becoming positive versus both genotypes (Fig 1).
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19 Seroconversion in animals from Group B was readily observed after 2-3 weeks after challenge
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21 against homologous antigen and remained well above detection limit till the end of the experiment.
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25 As expected, seroconversion against genotype E antigens was not generally observed with few time
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27 points close to the threshold limit (Fig 1a and 1c).
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30 Seroconversion against SU5 antigen reflected that to p16-25. Group A showed early seroconversion
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32 against both genotypes post-challenge while group B showed seroconversion exclusively against
33
34 CAEV-CO SU5-derived antigen.
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37 Seroconversion in goat kids was evaluated against genotypes B and E using both P16-25 and SU5
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39 ELISA tests employing either plasma or colostrum as sample source. Both ELISA tests showed a
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41 slow decrease of passive immunity without apparent seroconversion in both groups up to 16 weeks
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43 after birth (Fig. 2).
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Proviral load quantification

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50 The linear range of amplification of the duplex q-PCR was determined from 10^6 to 5 copies per
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52 reaction using Roccaverano and CAEV-CO plasmid dilutions. The amplification of the standard
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54 dilutions showed linearity over six orders of magnitude (1×10^6 to 10 copies/reaction) with a target
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1 specificity of 100%. The assay was able to amplify at least one replica of the standard dilution
2 containing 5 copies. The mean amplification efficiency of both targets was 97,0%.

3 The proviral load of both strains showed considerable variation during the time course of the
4 experiment. Considering the CAEV strain, according to figure 3, differences between animal groups
5 were slightly significant at 317 days post challenge (Wilcoxon Sum Rank test $p < 0.10$) and
6 progressively CAEV proviral load became significantly higher in animals belonging to the group B
7 than ones belonging to the group A during the three following time points (day#364 $p < 0.05$,
8 day#407 $p < 0.05$, day#462 $p < 0.05$). At the end of the experiment an increasing CAEV proviral load
9 in group A animals, resulted in non-significant differences between groups.

10 Four group A and seven group B goats gave birth to seven and eleven kids respectively. Colostrum
11 was sampled from the mothers and blood was collected from the kids during four months. None of
12 the kids born from group A goats carried the proviral CAEV genome, which was detected in three
13 of the eleven kids born to group B goats. This result could suggest a possible difference in CAEV
14 transmission to the progeny caused by the genotype E interference but, considering the small
15 sample size, no statistical analyses can be carried out.

36 37 *T cell proliferation*

38 As previously shown, infection with genotype E resulted in a genotype specific T cell proliferation
39 [10]. Challenge with CAEV-CO induced a broader response showing positive reactions versus both
40 genotype derived antigens (data not shown).

41 Infection with CAEV-CO (group B) induced similarly a strain specific response with no detectable
42 reaction against genotype E derived antigen.

43 44 45 46 47 48 49 50 51 52 53 54 *Clinical and histopathological examination*

55 Arthritic pathological score, expressed as the ratio between circumferences of the right carpal joint
56 and the contralateral metacarpal one, was calculated for each animal at each time point. All animals
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showed ratio levels below 1.8 throughout the whole experiment and no differences were recorded among groups (Wilcoxon Sum rank test $p > 0.05$ at each time point).

Right carpal joints (inoculation site) were generally more affected than left ones as expected. Precisely, in 5 goats belonging to the group A, lesions in the right synovial membrane samples were more severe than in the left, while in 2 animals no difference was observed. In the group B, 2 goats had right synovial membrane lesions more severe than those present in the left synovial membrane, while in 4 animals no difference was observed. No synovitis was observed in both right and left carpal joints of the group C animals (Table 2). Two out of three animals from group A showing severe lesions in the right carpal joint displayed no lesion in the left. In group B this tendency was observed only from severe to low degree of lesion (Table 2). In two cases (2631 and 2634) the comparison between the left and the right synovial membrane samples was not possible because the synovial lining cell layer was not adequately present in two out of the four samples collected from each animal.

Discussion

Attenuated virus vaccines are currently used for the prevention of a wide range of viruses such as Influenza virus [18], SIV [19], EIAV [20], chicken pox or yellow fever [21] conferring different degrees of protection. Among the strategies applied to immunize against HIV or SIV lentiviral infections, those based on live attenuated viruses have reached the highest levels of protection [22]. There are also several examples of effectiveness within lentiviral animal infections such as Feline immunodeficiency virus (FIV) and Equine infectious anemia virus (EIAV). FIV vaccines based on modified attenuated live viruses have been compared with subunit vaccines delivered as plasmid DNA or as protein, inducing moderate levels of cellular immunity and significant antibody responses conferring increased protection rates [23]. Regarding EIAV, among the various strategies explored, including immunizations based on attenuated viruses, inactivated virus particles, protein subunits, DNA vaccines, and live vectors, the highest level of protection was achieved when using

1 attenuated viruses, likely due to the continuous antigen exposure and optimized maturation of the
2 immune response. However, there is an inverse relationship between the level of protection and the
3 level of attenuation, indicating that a minimal replication rate is needed for eliciting protective
4 immune responses [21]. In the early 70's an attenuated strain (DLV120) obtained by in vitro
5 passages in donkeys, conferred protection against EIAV challenge [24], and was extensively used in
6 China with promising results [20].
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12 Immunization against SRLV has been explored deeply in the last decades with occasionally
13 disappointing results. Various strategies have been applied including artificially attenuated whole
14 virus [25-27], subunit vaccines supplied as protein or as expression plasmids or recombinant viruses
15 [28-31]. CAEV deletions of vif, essential for viral replication [32], induced weak responses with no
16 protection whereas CAEV tat-, replication competent [33] conferred some degree of protection but
17 still induced inflammatory lesions, further confirming the inverse relationship between attenuation
18 and protection [34].
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30 Early approaches within DNA immunization were carried out with env and tat encoding plasmids
31 and conferred some degree of protection related to Th1-biased response [35-37]. The newest
32 approaches employed, in a series of vaccination experiments, DNA plasmids encoding gag and env
33 viral genes and recombinant modified Vaccinia Ankara as inocula together with the employment of
34 immunologic adjuvants such as IFN-gamma or B7 costimulatory molecules [31]. Immunity was
35 stimulated enough to control viremia and proviral load in tissues but not in terms of reduced lesion
36 development, with an increased inflammation in target tissues, probably masking a competent
37 immune response [28, 29, 31]. These SRLV vaccine experiments confirmed that virus-specific
38 immune responses are a double edged sword that can contribute to either control or enhance virus
39 replication or disease. In all previous vaccination studies, a common experimental design was the
40 antigenic homology between vaccine antigen and challenge strain, leading to conclude that humoral
41 response, with different degree of stimulation recorded in the different vaccine strategies, may play
42 a deleterious role in protection.
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1 In this study, we infected goats with the naturally attenuated strain Roccaverano from the genotype
2 E of SRLV, in order to induce protection against both the proviral load and the development of
3 SRLV-related pathology. Goats were double infected with Roccaverano and CAEV-CO strains with
4 an interval of 4 months (Group A) or single-infected with CAEV-CO (Group B). Serological
5 analysis showed that, although a normal antibody response was elicited in both groups according to
6 the strains used, a clear type specific reactivity was obtained, being the pre-challenge of group A
7 reactive exclusively against genotype E antigen, becoming reactive in the post-challenge period
8 against both antigens. Group B as expected showed reactivity against genotype B antigen. Similar
9 results were obtained in lymphoproliferation assay, leading to suppose that, if any protection
10 occurs, this may not be related to a Th2 response, which is thought to be non-effective to control the
11 infection [30, 38].

12 Goats infected with genotypes E and B indeed showed lower proviral loads than goats single
13 infected with genotype B strongly indicating that the Roccaverano infection may have induced a
14 sustained immune response able to control genotype B proviral load and possibly, target tissues
15 colonization. Proviral load and lesion development, have been associated in a wide number of
16 SRLV infection studies [16, 39-41]. Assuming this relationship, histopathological results were in
17 line with proviral load ones, since dissemination of lesions was lower in Roccaverano infected
18 animals. Even so, macroscopic lesions (arthritic pathological score) evaluation did not show evident
19 differences. Explanations for this lack of contralateral affectation in group B, may include
20 differences between reference strain CAEV-CO and field strains causing arthritis in Roccaverano
21 goats.

22 Infection with Roccaverano and CAEV-CO strains induce exclusively homologous antibody and T
23 proliferative responses, but conferred protection against heterologous infection likely due to CTL
24 activity as our previous results strongly suggest [10].

25 Finally, we provided clear evidence that both viruses and antibodies against both SRLV strains
26 passed to the progeny, although genotype B was not detected in kids raised by group A mothers

(Fig 2; Table 3). This decreased viral flow to the progeny could reflect the lower proviral load found in the mothers, thus reducing viral transmission. Probably due to the short period of observation of the kids, we had not chance to evaluate the seroconversion against the viruses, but we only detected the decreasing of maternal antibody response.

The main goal of a vaccine is to achieve protection not only towards homologous strains but also against heterologous infection, this goal would be even more important in controlling lentiviral infections including SRLV, that are probably the most widely distributed lentivirus and one of the most heterogenous. Genotype E Roccaverano strain infection could be of extensive application not only in naïve goats but also in goats already infected with pathogenic genotype B. Roccaverano infection may open new approaches to naturally immunize against SRLV being the first naturally attenuated vaccine conferring protection in terms of reduced viral load and, possibly, reduction of viral colonization and lesion development.

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Figure 1. Seroconversion to type specific antigens. Seroconversion against SRLV genotype E Roccaverano strain (a: p16-25 antigen; c: SU5 antigen) and against SRLV genotype B CAEV-Co strain (b: p16-25; d: SU5) in animals belonging to the group A (circles and solid line) and to the group B (squares and dashed line). Seroconversion is indicated as the average of reactivity among animals and it is expressed as the percentage against the homologous positive control. Vertical solid lines represent the standard error of the mean, calculated among animals belonging to the same group and within the same collection time. Vertical dotted line represents the time of challenge (day=133).

Figure 2. Seroconversion in the progeny. Seroconversion against SRLV genotype E Roccaverano strain (a: p16-25 antigen; c: SU5 antigen) and against SRLV genotype B CAEV-Co strain (b: p16-25; d: SU5) in kids born from animals belonging to the group A (circles and solid line) and to the group B (squares and dashed line). Seroconversion is indicated as the average of reactivity among animals and it is expressed as the percentage against the homologous positive control. Vertical solid lines represent the standard error of the mean, calculated among animals belonging to the same group and within the same collection time.

Figure 3. CAEV Proviral load. Mean number of provirus copies in blood of animals belonging to the group A (circles and solid line) and B (squares and dashed line). Vertical solid lines represent the standard error of the mean, calculated among animals belonging to the same group and within the same collection time. Vertical dotted line represents the time of challenge (day=133). Statistical differences are reported on the x axis (‘°’ Wilcoxon Rank Sum test $p < 0.10$; ‘*’ Wilcoxon Rank Sum test $p < 0.05$)

1 **Table 1.** Primers and probes

Primer or probe	Sequence (5'-3')	Nucleotide position	Amplicon length (bp)
Roccaverano_Fw	AACACAGGAAGAGAAATAGTGAGG	3863-3886	
Roccaverano_Rev	GAGCGATCTTGCATCTTGGTG	3976-3956	114
Roccaverano_Probe	<i>FAM-CCAGTGTCTTCTTCCGCCTCCGG-BHQ-1</i>	3949-3926	
CAEV-Co_Fw	AAAGAATGCAGAGGAAAGAGAGAC	1763-1786	
CAEV-Co_Rev	GGTGCTGAAGTTATTCCATAGGAG	1868-1845	106
CAEV-Co_Probe	<i>HEX-CGGACGGCACCCACGTATCCC-BHQ-1</i>	1841-1820	

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4 **Table 2.** Synovitis observed in right (R) and left (L) carpal joints.

group	animal n.	right (R)	left (L)	Comparison R vs L
A	50011	low	no	>
A	2638	moderate	low	>
A	2629	severe	low	>
A	2633	severe	no	>
A	2634	unclassified	moderate	-
A	50013	low	low	=
A	2588	low	low	=
A	50010	severe	no	>
B	2637	low	low	=
B	2635	severe	low	>
B	50015	low	low	=
B	50012	severe	low	>
B	50014	low	low	=
B	2631	moderate	unclassified	-
B	2587	low	low	=
C	7686	no	no	=
C	7837	no	no	=
C	7841	no	no	=

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7 **Table 3.** Proviral load of Roccaverano (RV) and CAEV viral strains in mothers, colostrum and
 8 kids.

Group	Goat N°	Virus	Viral load (Wk0 to Wk51)										Colostrum	Kids			
A	2588	RV	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+
		CAEV	-	+	+	-	+	+	-	-	-	-	+	-	-	-	-
	2633	RV	+	+	+	-	-	+	-	+	+	+	+	-	+	-	
		CAEV	+	+	-	-	-	+	-	+	-	+	-	-	-	-	
	50010	RV	+	+	+	+	+	+	+	+	+	+	+	-	-		
		CAEV	-	-	-	+	+	+	-	+	-	-	-	-	-		
	50011	RV	+	-	+	+	+	+	+	+	+	+	+	+	+		
		CAEV	-	-	-	+	-	-	-	+	-	-	+	+	-		
B	2587	CAEV	nd	+	+	+	+	+	+	+	+	+	-	-	+		
	2631	CAEV	nd	+	+	-	+	+	+	+	+	+	-	-	-		
	2635	CAEV	nd	+	+	+	+	+	+	+	+	+	-	-			
	2637	CAEV	nd	+	-	-	-	-	-	-	+	-	+	-	-		
	50012	CAEV	-	-	+	+	+	+	+	+	+	+	+	+	+		
	50014	CAEV	-	+	-	-	-	+	-	+	-	-	-	-	-		
	50015	CAEV	-	+	+	-	+	+	+	+	+	+	+	-	-		

Figure 1
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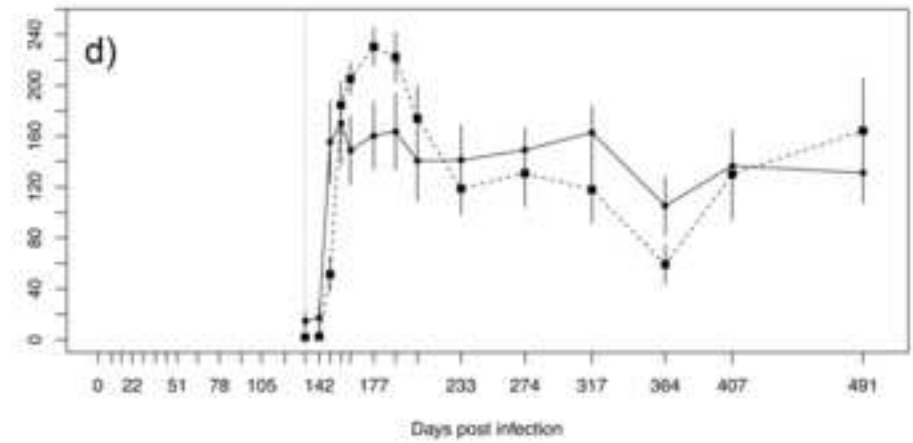
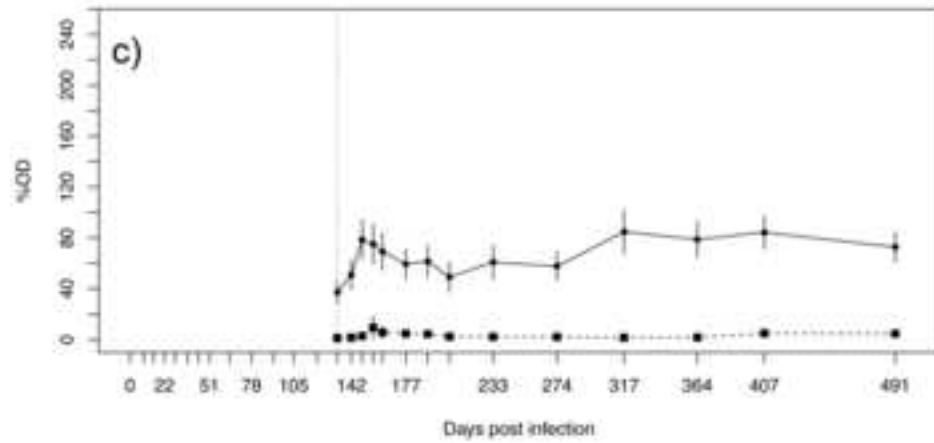
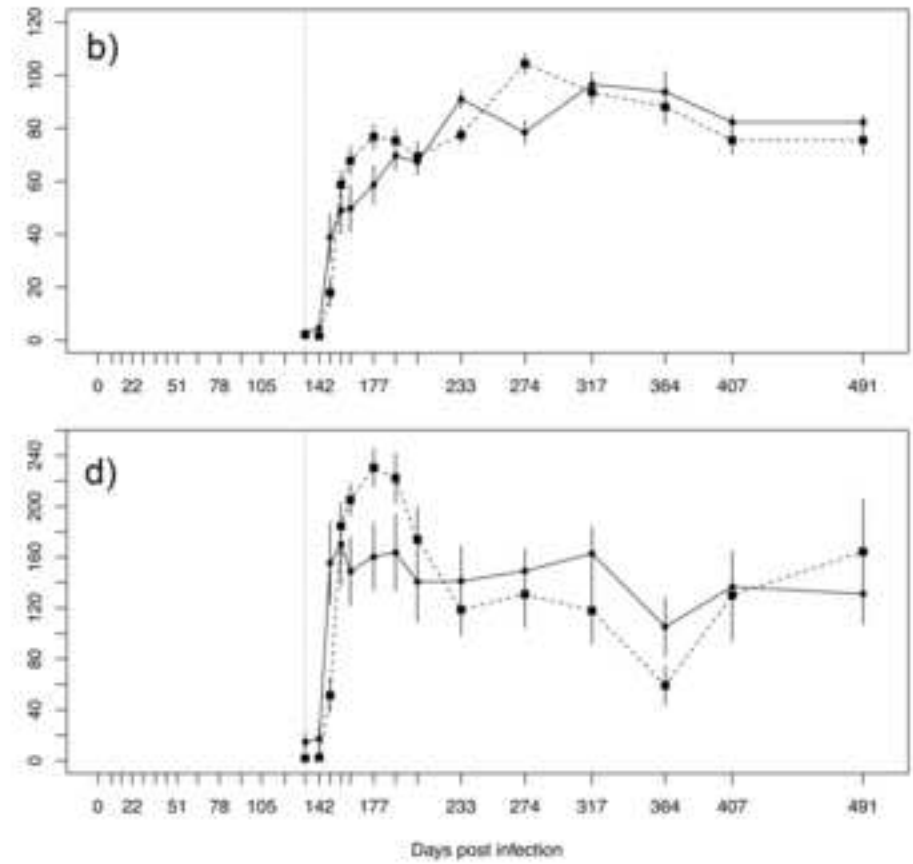
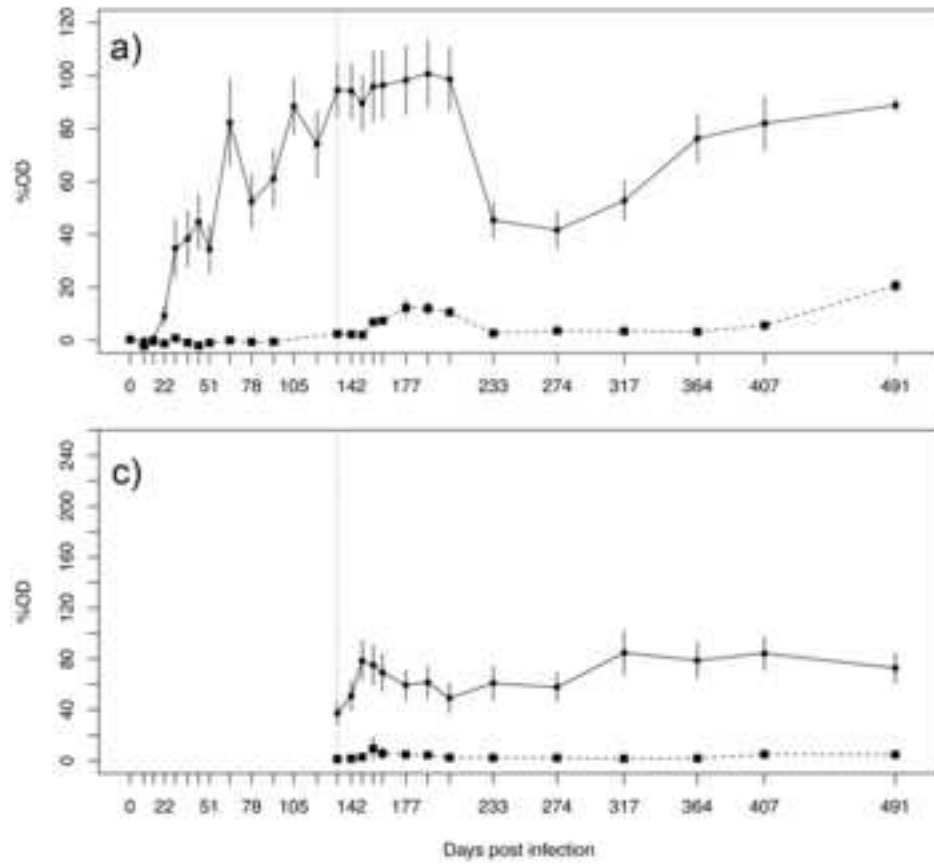


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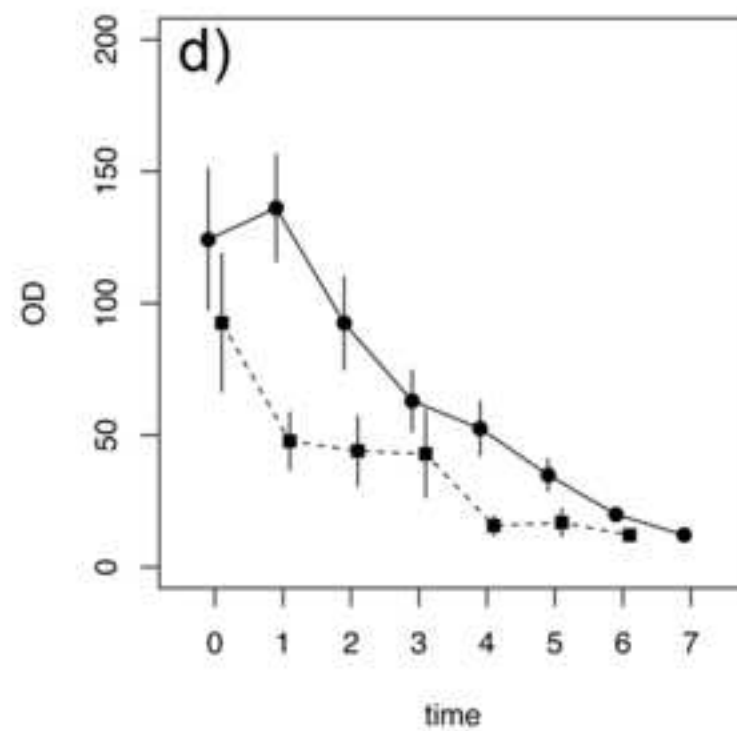
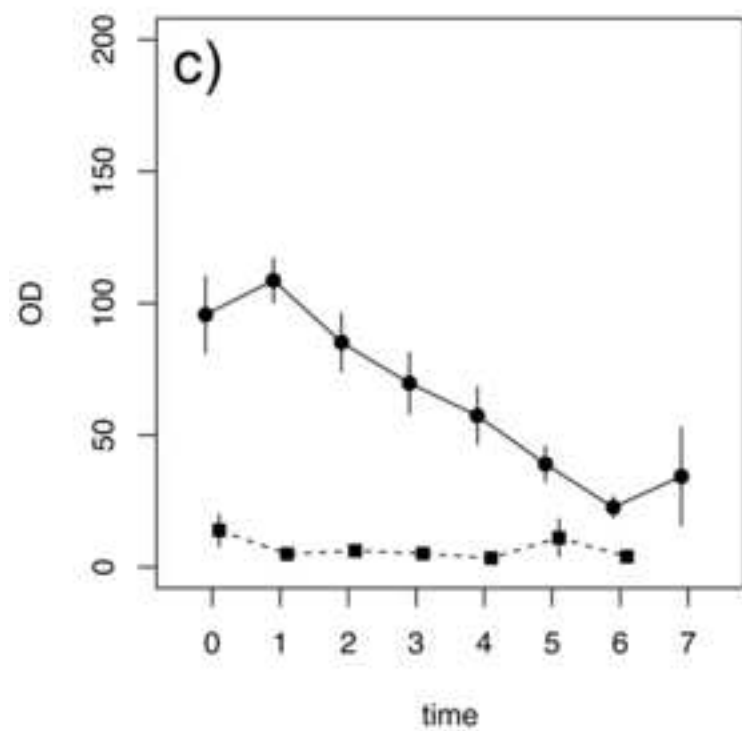
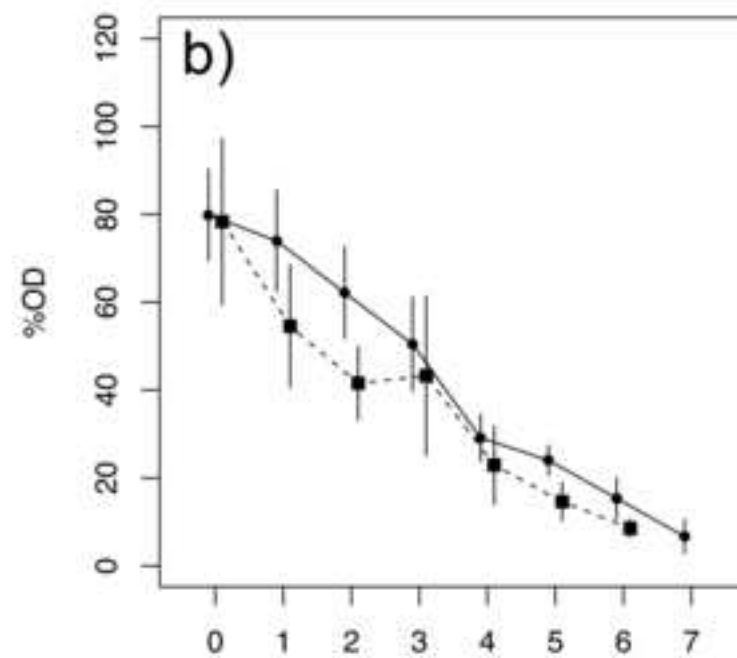
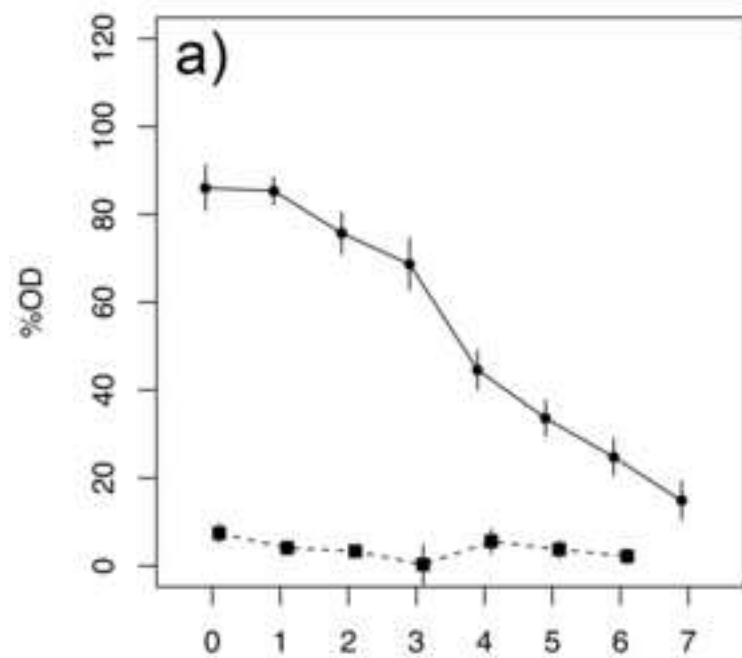


Figure 3
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