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Serological characterization of the new genotype E of small ruminant lentivirus in roccaverano goat flocks

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Abstract

Maedi visna virus (MVV) and caprine arthritis encephalitis virus (CAEV) are a heterogeneous group of infectious agents affecting sheep and goats. Due to their natural cross-species infection they are referred to as small-ruminant lentiviruses (SRLV). Recently a new genetic cluster, highly divergent from MVV and CAEV was identified in the north-west part of Italy. A panel of genotype E specific antigens was developed and evaluated in flocks infected with B1 and E strains. The results clearly indicate that a strain specific antigen is required to correctly identify animals infected with different genotypes.

Keywords: ELISA, Genotype E, Small ruminant lentivirus

Introduction

Small ruminant lentiviruses cause persistent infections and chronic debilitating diseases in goats and sheep all over the world. There are four main genetic clusters of SRLV: maedi visna virus (MVV), mainly genotype A; caprine arthritis encephalitis virus (CAEV), mainly genotype B; genotype C Norwegian isolates from arthritic goats; and genotype D, only identified in Switzerland and Spain. Recent phylogenetic studies carried out in Italy have shown no species barrier between strains of caprine and ovine origin. In fact, genotypes previously found in sheep have been recently identified in goats (subtypes A1, A8, A9) and vice versa (subtypes B1 and B2). The presence of a new SRLV cluster (genotype E) in goats of the Roccaverano breed has been recently described in Italy. Antigenic divergence between genotype E and classical A and B SRLV prototypes could suggest antigenic escape to routine diagnostic tests employing mainly A or B-type antigens (Rosati et al. 1999; Grego et al. 2005; Lacerenza et al. 2006).

In this study we have developed a new panel of specific recombinant antigens derived from genotype E and evaluated the serological response to homologous and heterologous antigens in genetically characterized flocks.

Materials and methods

The area of study is situated in the north-east of Italy, Piedmont region. Three flocks from the Roccaverano breed were selected on a serological basis in a previous study using an indirect ELISA based on recombinant p25-TM antigen (Rosati et al. 2004).

Buffy coats were also collected, DNA extracted and analysed by nested PCR amplifying a 800 nt region of the gag gene (Grego et al. 2007). Positive samples were sequenced to identify the circulating genotype. Depending on the sequence, a second indirect ELISA based on recombinant p16-25 fusion protein was developed as previously described (Lacerenza et al. 2006). Briefly, the gag region encoding for p16 and p25 proteins was amplified, using a strain-specific set of primers, and cloned into pGEX-6his in fusion with the glutathione S-transferase (GST) gene. Recombinant antigens were purified by a two-step process involving affinity chromatography and GST cleavage with a site-specific protease. The purity and concentration of the eluted antigens were assessed by SDS-PAGE and the Bradford protein quantification system, respectively. Sera collected from the same flocks were then tested using genotype-specific fusion antigens and specific reactivity differences were estimated using the t-Student test and Wilcoxon test for paired samples.

Results

Flocks were classified as NG in which genotypes B1 and E were present; TA in which only genotype B1 was identified; and flock BL in which only genotype E was observed.

Sera from the three complete flocks were tested using p16–25 indirect ELISA based on genotypes B and E. In flocks infected with a single genotype (flocks BL and TA) (Figs. 1 and 2) the homologous antigen absorbance was significantly higher (t-test $p < 0.0001$; mean of differences >1.28) than that observed for the heterologous antigen (Fig. 3). In the case of co-infection (flock NG), absorbance differences between the homologous and heterologous antigens, are still significant (Wilcoxon test $p < 0.0001$) but the mean between differences is lower compared with single infected flocks. This is most likely due to the different distribution of infection within the flock. Several samples classified as infected by genotype E tested negative to the p25-TM screening test, confirming genotype E antigenic divergence compared to commercially available serologic tests. This could result in misdiagnosis of this genotype in the small ruminant population.

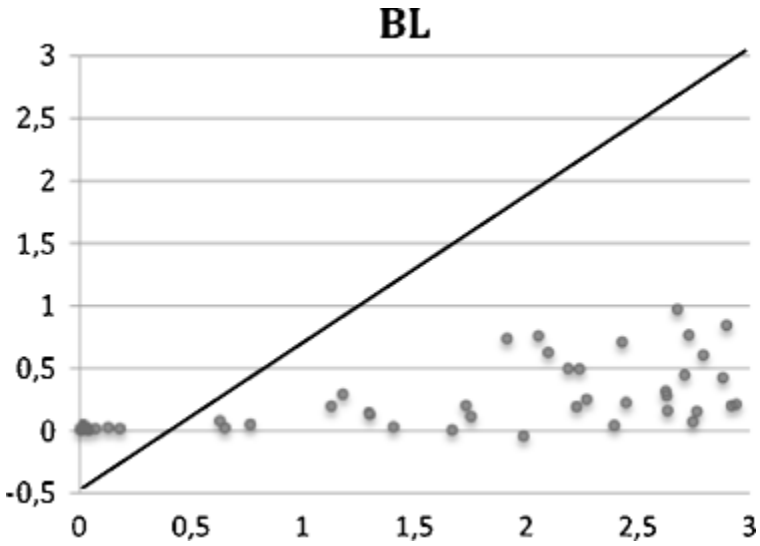

Fig. 1

p16–25 (genotypes B and E) indirect ELISA absorbance (450 nm) distribution in genotype E infected flock (BL)

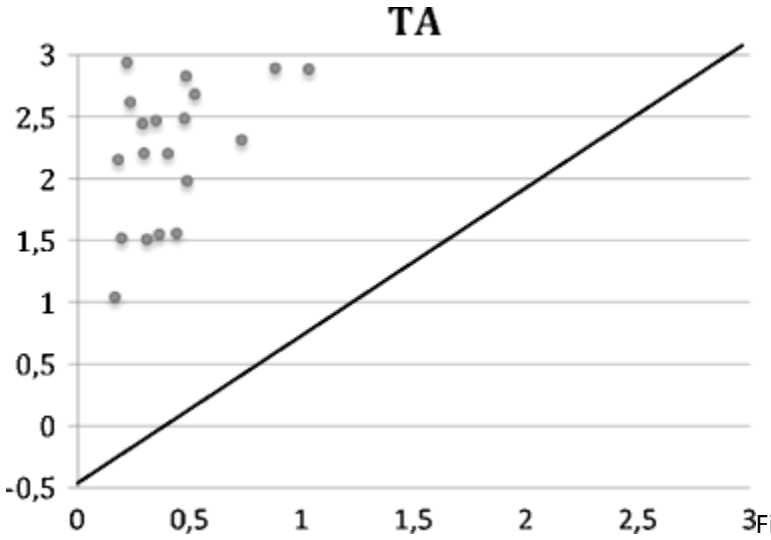

Fig. 2

p16–25 (genotypes B and E) indirect ELISA absorbance (450 nm) distribution in genotype B infected flock (TA)

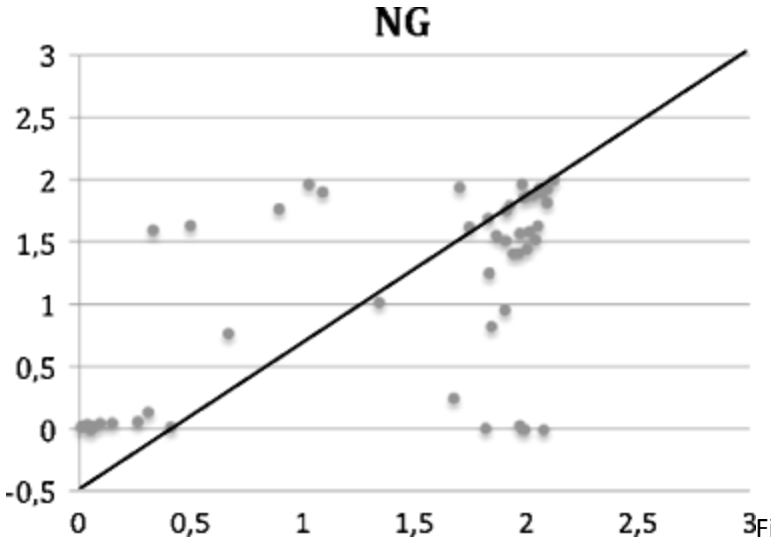

Fig. 3

p16–25 (genotypes B and E) indirect ELISA absorbance (450 nm) distribution in coinfected flock (NG)

Discussion

The complete sequence of a genotype E derived strain has recently been described, resulting in a genetic similarity not higher than 70% compared with classical SRLV isolates.

Antigenic diversity involves the main epitopes present in the structural proteins widely used in SRLV diagnosis. In this study, we used cross-reactive gag antigens derived from MVV and CAEV sequences. Recent studies have shown that antigenic diversity may ensure early diagnosis using homologous rather than heterologous derived antigens. Since the antigenic diversity of genotype E is higher than between MVV and CAEV strains, it could be argue that serologic reactivity will be genotype-specific. Mixed absorbance distribution against genotypes B and E in NG flock (Fig. 1), was not unexpected since by sequence analysis of PCR amplicons, both genotypes were detected and, consequently, co-infections are a usual feature in this flock. In contrast, maximal serologic reactivity against homologous antigen was observed in flocks BL and TA, in which sequence analysis showed a single circulating strain.

The description of the novel field strain belonging to genotype E, highlights the problem of the current routine serologic methods. Tests based on a single strain could select escape mutants to available serologic

tests. Flocks under study had adhered to a SRLV control program, that could has selected particular strains such as genotype E, being misdiagnosed by commercial tests as confirmed by its high antigenic diversity. Furthermore, the genotype-specific ELISA p16–25 test has been shown to be a practical and economic serologic marker for serotyping circulating strains within a population.

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