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Development of specific diagnostic test for small ruminant lentivirus genotype E

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ABSTRACT

Small ruminant lentivirus (SRLV) belonging to the highly divergent genotype E has recently been identified in the Italian goat breed Roccaverano. In this report we have developed a specific serological test based on recombinant matrix/capsid antigen fusion protein. Performance has been evaluated and compared with a similar test based on genotype B antigen. Herds under study were selected according to the infectious status characterized by blood PCR and sequencing. Results clearly showed that B and E based recombinant ELISA only detected homologous infection and an apparent cross-reactivity was recorded in a herd in which co-infection was present. Three commercially available ELISAs showed different abilities in detecting genotype E infection, being the whole virus-based immunoassay the best choice. Genotype E-recombinant antigen was not detected in ELISA by three commercially available Mabs known to be cross-reactive among CAEV and MVV capsid antigens, further supporting the high divergence of the E genotype from others. Finally, a SRLV-free herd according to commercial ELISA testing, was analysed in the same area where genotype E was identified and few animals belonging to Roccaverano breed were found slightly reactive with the E antigens. Our results suggest that the prevalence of genotype E in other small ruminant populations may be conveniently estimated using a comparative assay based on a combination of genotype specific recombinant antigens and may highlight a wider space in which SRLVs evolve.

1. Introduction

Small ruminant lentiviruses (SRLVs) are a heterogeneous group of viruses affecting sheep and goat and are responsible for chronic debilitating diseases known as Maedi Visna (MV) and caprine arthritis-encefalitis (CAE) (Pepin et al., 1998). Viral isolates characterized so far, show different genetic, antigenic and biological properties and are no longer considered species-specific (Pisoni et al., 2005; Shah et al., 2004b). From an antigenic point of view, most SRLVs can be classified as MVV-like or CAEV-like, corresponding to genotype A and B respectively (Shah et al., 2004a). Previous studies have suggested that early serological diagnosis can be achieved using homologous antigen (Lacerenza et al., 2006). Nevertheless, most of the currently available diagnostic tests are produced using a single strain-based antigen preparation which is believed to detect cross-reacting antibodies against epitopes located in structural proteins (Gogolewski et al., 1985). Recently, a novel genotype E has been identified in the local breed Roccaverano in north-west Italy. First sequences were obtained by chance in a caprine herd, using a set of degenerated primers designed to amplify a *gag* fragment from the majority of known genotypes, encompassing major linear capsid

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antigen epitopes. Following a preliminary sequence screening, it seemed quite clear that this viral cluster might have escaped diagnosis in the field using conventional antigen preparations, likely due to the low similarity found in the major immunodominant regions (Grego et al., 2007). A viral strain was subsequently isolated from an apparently healthy goat highly reactive by ELISA against genotype E-derived major capsid antigen epitopes (Reina et al., 2009). Genetic features of this genotype have been described in three epidemiologically unrelated herds. The complete genome (~8.4 Kb) presented two major deletions corresponding to the dUTPase subunit of the *pol* gene and to the *vpr* accessory gene. Based on previous studies in which such subunits were independently deleted from a pathogenic infectious clone, these deletions could explain why the viral cluster is not related to any known clinical signs, representing a natural, well host-adapted, low pathogenic lentivirus (Harmache et al., 1996; Turelli et al., 1996; Zhang et al., 2003). Moreover, preliminary epidemiological data suggest that infection can persist in the population through familiar lineage, with a low tendency to spread horizontally. To date, no information is available on the prevalence of genotype E in larger goat populations due to the lack of a specific antibody detection system. To address this issue, in this report we have developed genotype E specific recombinant antigens which were tested with a panel of monoclonal antibodies (Mabs) known to be reactive against CAEV and MVV capsid antigens. These genotype E derived antigens were also used to develop an indirect ELISA in order to test a panel of goat sera belonging to herds in which the SRLV infectious status was determined by PCR product sequence analysis. Serological and sequence data were in agreement highlighting the importance of using genotype specific tests when determining SRLV seroprevalence, assessing SRLV-free status and searchng for epidemiological information.

2. Material and methods

2.1. Virus and plasmids

Roccaverano strain was originally isolated using mammary gland explants from an adult goat and a complete proviral sequence was obtained (Genbank accession number EU293537). The *gag* gene was amplified by concatenating overlapping PCR fragments and cloned in pCRTopoXL (Invitrogen). The gene fragment coding for Matrix (P16) and major Capsid Antigen (P25) was subsequently amplified and was subcloned between the *BamH1/EcoR1* sites of pGEX6His following site-directed PCR-mediated mutagenesis suppressing an internal *EcoR1* restriction site. This plasmid, derived from pGEX6P prokaryote expression vector (GE Healthcare), was modified by inserting an in frame 6Xhis-tag between *EcoR1* and *Sal1* restriction sites thus allowing a double step affinity purification.

2.2. Expression and purification of P16–25 recombinant antigens and P25-B3 subunit epitopes

Transformed *E. coli* BL21 bacteria were induced at early log phase for 2 h with 0.5 mM IPTG under agitation. Bacterial cells were recovered by centrifugation and lysed by physicochemical methods. Recombinant GST/P16–25/6H fusion protein was recovered in the soluble fraction and the first affinity step was carried out in batch using glutathione Sepharose 4B (GE Healthcare). GST cleavage was achieved in pooled eluted fractions using PreScission Protease (2 U/mg) (GE Healthcare). Solution containing GST and P16–25/6H was dialyzed for 24 h to remove reducing and chelating agents and loaded into a Hi-Trap chelating HP column (GE Healthcare), positively charged with nickel ions. Following immobilised metal chelate affinity chromatography, purity and yield of recombinant antigen was estimated by SDS–PAGE and DC protein assay (BioRad).

Using the same protocol, the same gag antigens derived from strain It-Pi1 (genotype B) were employed to generate the antigenic CAEV-like counterpart.

Subunit immunodominant capsid antigen epitopes of MVV and CAEV had previously been characterised. In this study a third version using genotype E (P25-B3) derived sequence was produced, generating a GST fusion protein as previously described (Grego et al., 2002; Rosati et al., 1999).

2.3. Blood samples, polymerase chain reaction and sequencing

Twelve caprine herds were selected in this and in a previous study (Grego et al., 2007). Heparinized blood samples were obtained from a number of adult animals, representative of each herd: and DNA was extracted from white blood cells using DNA blood kit (Qiagen). A *gag* nested PCR previously developed (Grego et al., 2007) and known to detect the highest number of SRLV genotypes/subtypes was applied to each sample and all positive results with suitable bands were sequenced. After, this preliminary screening, five herds of Roccaverano breed were selected: a first herd, BL ($n = 52$) in which only genotype E was detected; a second herd, NG ($n = 40$) in which genotype B and E were detected; and a third group of goats, TM ($n = 20$), BM ($n = 18$) and CF ($n = 6$) in which only genotype B was present. In this study, all caprine herds were retested when possible at completion and additional sequences were obtained.

To date, the presence of genotype E had been limited to few herds of the Roccaverano breed. Therefore, an additional three-breed long term SRLV negative herd ($n = 400$) was also included for serological testing. The herd consisted of 109 Roccaverano, 107 Saanen and 184 French Alpine goats.

2.4. Serum samples and ELISAs

For P16–25 recombinant ELISA, microplates (Immunomaxi TPP) were coated with 100 ng of P16–25 derived from B and E genotypes or water as negative antigen (Fig. 1). Plates were allowed to dry overnight at 37 °C and then blocked with 2.5% bovine casein for 1 h at 37 °C. After four washes, serum samples were diluted 1/20 in phosphate-buffered saline containing 1.25% casein and incubated for 1 h at 37 °C. Subsequently to the washing step, anti-sheep/