Small ruminant lentiviruses in Jordan: evaluation of sheep and goat serological response using recombinant and peptide antigens

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Abstract

Small ruminant lentiviruses infect sheep and goats worldwide, causing chronic progressive diseases and relevant economic losses. Disease eradication and prevention is mostly based on serological testing. The goal of this research was to investigate the presence of the small ruminant lentiviruses (SRLVs) in Jordan and to characterize the serological response in sheep and goat populations. A panel of sera were collected from flocks located in Northern Jordan and Jordan Valley. The samples were tested using three ELISA assays: a commercial- ly available ELISA based on p25 recombinant protein and transmembrane peptide derived from British maedi–visna vi- rus (MVV) EV1 strain, an ELISA based on P16-P25 recom- binant protein derived from two Italian strains representative of MVV- and caprine arthritis encephalitis virus (CAEV)-like SRLVs, and an ELISA based on SU5 peptide from the same two Italian isolates. The results indicate that both MVV- and CAEV-like strains are present in Jordan and that the majority of the viruses circulating among sheep and goat populations belong to the MVV-like genotype.

Keywords Small ruminant lentiviruses . Jordan . ELISA . Serotyping

Introduction

Maedi–visna virus (MVV) and caprine arthritis encephalitis virus (CAEV) are non-oncogenic retroviruses belonging to the genus Lentivirus. They have long been regarded as distinct viruses, restricted respectively to ovine and caprine hosts. However, as cross-species transmission has been increasingly demonstrated during the last two decades (Zanoni 1998; Shah et al. 2004; Pisoni et al. 2005), the term small ruminant lentiviruses (SRLVs) is presently used to refer to both viruses.

SRLVs cause persistent infections characterized by a long incubation period, which result in chronic and progressive disorders involving the lungs, joints, mammary gland, and central nervous system. Therapy and effective vaccines are not yet available (González et al. 2005; Pétursson et al. 2005; Nenci et al. 2007; Torsteinsdóttir et al. 2007; Reina et al. 2008; Niesalla et al. 2009; Blacklaws 2012); therefore, eradication and prevention largely depend on early identification of infected animals. This is routinely done by serological analy- sis, with the ELISA assay being the most suitable technique for large-scale screening. Several ELISAs have been developed so far, mostly based on antigens derived from a single viral strain. The specificity of these assays is generally high, but the sen- sitivity shows extensive variability due to the antigenic heterogeneity of SRLVs (Herrmann-Hoesing 2010), which reflects the high genetic variability of this group of viruses, presently classified into five genotypes, A to E (Shah et al. 2004; Grego et al. 2007). A and B are the most common and widely distributed genotypes: genotype A, which includes MVV-like isolates, is highly heterogeneous, with 11 subtypes of ovine and caprine origin; genotype B comprises CAEV-like isolates, with three subtypes so far described (Bertolotti et al. 2011).
SRLV circulation has been reported worldwide with few exceptions: in Middle East, the presence of MVV and/or CAEV has been documented in Saudi Arabia, Syria, Palestine, Israel, and Turkey (Alluwaimi et al. 1990; Giangaspero et al. 1993a, b; Burgu et al. 1994; Hananeh and Barhoom 2009; Leitner et al. 2010; Preziuso et al. 2010; Muz et al. 2013). In Jordan, the circulation of CAEV in the goat population has been demon- strated with an ELISA assay based on the surface (SU) subunit of the ENV protein derived from a B1 North American CAEV strain (Al-Qudah et al. 2006). The present study investigated the prevalence of SRLVs in sheep and goat populations at flock level and in individual animals in Northern Jordan and Jordan Valley. Since the genetic and antigenic background of the SRLVs circulating in Jordan was not yet characterized, a set of ELISA assays was used to analyze sheep and goat sera, to increase the overall sensitivity of the investigation and characterize the antibody response of infected animals.
The assays were based on GAG and ENV antigens de- rived from MVV and CAEV isolates of different geographical origin and genotype.

Materials and methods

Study population and sample collection

Sampling was performed in two areas of Jordan, namely Northern Jordan and Jordan Valley. Northern Jordan, the region stretching from Amman in the South to the Yarmuk River and the Syrian border on the north, is the most fertile region of the country. The altitude is in the range of 500– 1,200 m a.s.l., springs abound, and streams flow through the valleys from fall through spring. The climate is temperate, with temperatures varying between −4 and 8 °C in winter and 29 to 41 °C in summer and the rainfall varies between 100 and 600 mm. Flocks graze from spring to early summer and are supplemented with concentrate for the rest of the year. Lambing season lasts from November until April. The Jordan Valley lies between 200 and 400 m below sea level, extending from Lake Tiberias to the Dead Sea; it is sur- rounded in the East and West by high mountains. Jordan Valley has a warmer climate with rainfall decreasing from approximately 300 mm in the north to 102 mm in the south and the period of grazing is possible in fall and winter but may be limited during the dry season due to water shortage. Flocks feed often on the remains left on the ground after the harvest. Sheep and goat flocks are managed under free- range nomadic or semi-nomadic husbandry and change location depending on the season. Goats and sheep are often housed in the same areas and

will graze together as a mixed flock. The majority of the sheep analyzed belonged to Awassi breed with some crossbreed; the majority of goats were of local Baladi breed with only few pure Shami goats. The age of the sampled animals was over 1 year. None of the goats presented signs of arthritis, while several sheep pre- sented symptoms of chronic pneumonia and, in few cases, symptoms of chronic mastitis (hard udder).
Whole blood samples were collected from 231 sheep and 203 goats belonging to 28 flocks, five located in Northern Jordan and 23 along the Jordan Valley, accounting for a total of 1,615 sheep and 813 goats. Seven flocks were mixed; the remaining consisted exclusively of either sheep (10 flocks) or goats (11 flocks). Serum samples prepared from whole blood were aliquoted and stored at −20 °C.

Serological tests

Since no data were available on the antigenic makeup of the SRLVs circulating in Jordan, in an attempt to increase the overall sensitivity of the investigation and to characterize the antibody response, the sera collected from sheep and goats were analyzed with ELITEST and P16-P25 assays, based on GAG and ENV antigens derived from MVV and CAEV isolates of different geographical origin and genotype. A flock was considered seropositive when at least one animal resulted positive to at least one of these serological assays.
ELITEST-MVV/CAEV (Hyphen Biomed, France) The assay uses as antigens a recombinant p25 capsid protein encoded by the GAG gene and a peptide corresponding to an immunodominant region of the transmembrane (TM) protein gp46 encoded by the ENV gene, derived from the British MVV EV1 isolate, belonging to genotype A1. The assay was shown to be able to detect antibodies to SRLVs in sheep and in goats. The overall sensitivity of ELITEST was estimated as 99.4 % (95 % confidence interval (CI), 98.4–99.8 %) and specificity as 99.3 % (95 % CI, 98.7–99.6 %) (Saman et al. 1999). The serum samples were examined according to the manufacturer's instructions. The absorbance was measured at 450 and 595 nm wavelength. To minimize intra- and inter-plate variations, the cutoff (CO) was calculated for each plate by introducing three positive and three negative control sera and expressed as $CO = (P-N)/4 + N$ where P is the mean absorbance of the positive controls and N is the mean absorbance of the negative controls.
Results were expressed as $R = S/CO$ where S was the optical density value of the sample. Samples were considered positive when R was more than 1. P16-P25 ELISA The assay is based on two recombinant P16-matrix/P25-capsid fusion proteins derived from Italian MVV-like isolate It-561 and CAEV-like isolate It-Pi1, be- longing to genotypes A9 and B2, respectively (Grego et al. 2005; Lacerenza et al. 2006). This ELISA assay differentiates the infecting viruses according to their antibody reactivity to either MVV- or CAEV-like antigens (Lacerenza et al. 2006).
Briefly, microplates were coated with 100 ng of each antigen per well. Water was used as a negative control antigen. Positive control consisted in a pool of positive sera which was equally reactive against It-561 and It-Pi1 anti- gens. Sera were diluted 1/20 and incubated for 1 h at 37 °C. After four washes, peroxidase conjugated anti-sheep/goat IgG monoclonal antibody (Sigma) was added and plates incubated for 1 h at 37 °C. After the final washing, 2,2'- azino-bis 3-ethylbenzothiazoline-6-sulphonic acid (ABTS, Sigma) was added and plates read at 405 nm wavelength. Sera having a reactivity higher than 40 % with respect to the positive control were considered positive. SU5 ELISA The SU5 ELISA was used to analyze all sera (166 sheep and 169 goat), collected from the seropositive flocks identified by the two assays described above.

The assay is based on a combination of two 25aa synthetic peptides encompassing the SU5 domain of the SU subunit of the envelope glycoprotein of Italian isolates It- 561 and It-Pi1 (Carrozza et al. 2009). All serum samples were first tested against a pool of the two SU5 peptides. The sera positive to the pool were further tested against each individual peptide. The assays were performed as previously described (Carrozza et al. 2009). ELISA plates were coated overnight at 37 °C with peptide antigens (50 µl/well) and incubated for 1 h at 37 °C with sera diluted 1/20 and for 1 h at room temperature with peroxidase-conjugate Mab anti-sheep/goat IgG (Sigma). Optical density was measured at 405 nm wavelength after 40 min incubation with ABTS (Sigma). A panel of six negative and two positive control sera were included in each plate. The cutoff was calculated for each plate as the mean absorbance of the negative sera plus 3 standard deviations.

Results

The serological survey performed with ELITEST and P16- P25 assays revealed that all flocks located in Northern Jordan, namely two sheep and three mixed, were seropositive; in Jordan Valley, seropositivity was found in 17 flocks out of 23, including all eight sheep, 7 out of 11 goat, and 2 out of 4 mixed flocks (Fig.1a), with an overall prevalence of 100, 71, and 63 % for the three types of flock, respectively. A total of 434 sera, 231 from sheep and 203 from goats, were analyzed and 84 sheep and 32 goat sera reacted to at least one test, with an overall seroprevalence of 26.7 % (Fig. 1b) and a seroprevalence of 36.4 and 15.9 % for sheep and goats, respectively (Table 1). Table 1 also shows the number of sheep and goat sera reacting only to one assay or to both. Briefly, a total of 15 sera reacted exclusively to ELITEST, 44 exclusively to P16-P25 ELISA, and 57 to both tests. This result highlights the usefulness of using more than one assay, especially when the antigenic background of the viruses circulating in the area under study is not known. If using only ELITEST, 44 infected animals would have escaped detection, while the exclusive use of P16-P25 would have missed 15 infected animals. This analysis also highlights a difference between sheep and goat sera reactivity: sheep sera reacted in nearly equal numbers to ELITEST, based on British MVV-like EV1 isolate, and to P16-P25 assay, based on Italian MVV-like It-561 and CAEV-like It- Pi1 isolates, whereas the large majority of goat sera reacted exclusively to Italian antigens. Furthermore, the genotype-specific P16-P25 assay revealed that both MVV and CAEV circulate among sheep and goat populations in Jordan and that the MVV type seems to be prevalent (Fig. 2a). In particular, 42 out of 71 (60 %) sheep and 26 out of 30 (87 %) goat sera positive to the assay reacted exclusively to MVV-like antigen and 28 out of 71 (39 %) sheep and 2 out of 30 (7 %) goat sera reacted to both MVV- and CAEV-like antigens. Only one (1 %) sheep and two (6 %) goat sera reacted exclusively to CAEV-like antigens. All sheep and goat sera collected from the seropositive flocks were examined by SU5 ELISA, with 16 sheep and 12 goat sera resulting positive. The responses to the individual SU5 antigens were in agreement with the results of the P16-P25 assay, with most sera reacting against the MVV-like epitopes (Fig. 2b).

Discussion

SRLV infections are present among sheep and goat populations worldwide and their prevalence in different countries has been extensively investigated with serological surveys. Data on SRLV infection in the small ruminant population of Middle East are limited to a few countries, including Syria, Palestine, Israel, Saudi Arabia, and Turkey (Alluwaimi et al. 1990; Giangaspero et al. 1993a, b; Burgu et al. 1994; Hananeh and Barhoom 2009; Leitner et al. 2010; Preziuso et al. 2010; Muz et al. 2013). In Jordan, the presence of CAEV in goat pop-

ulations has been demonstrated with an ELISA assay based on the SU subunit of the envelope protein derived from North American strain CAEV-63, belonging to genotype B1 (Al- Qudah et al. 2006). The present study investigated the reac- tivity of sheep and goat sera sampled in Jordan to antigens derived from SRLV isolates of different geographical origin and genotype, providing evidence that both MVV- and CAEV-like viruses circulate in the country. All sheep flocks resulted seropositive, whereas seroprevalence at flock level was 71 and 63 % in mixed and goat flocks, respectively. The overall seroprevalence in the tested animals was also higher for sheep than for goat sera. The prevalence determined at flock level and in individual animals was higher than the one observed by Al-Qudah et al. (2006). This may reflect the increased sensitivity reached when using a set of ELISA tests employing antigens derived from strains of different genotype, for the detection of viruses whose antigenic background is not known. ELITEST and P16-P25 ELISA identified nearly equal numbers of positive sheep sera, averaging about 83 % of the total, although the two groups were not fully overlapping. The difference was much more pronounced among goats, with ELITEST and P16-P25 being able to detect 12.5 and 94 % of the positive sera, respectively. These results presumably reflect the differences between the antigens employed by the two tests, therefore their different ability to be recognized by the antibodies produced in response to the Jordanian SRLVs: the antigens used by ELITEST were derived from British isolate EV1, belonging to genotype A1, and P16-P25 ELISA is based on antigens derived from Italian isolates It-561 and It- Pi1, classified as genotype A9 and B2, respectively. In addition, TM epitope present only in the former test and matrix protein in the latter may have revealed an exclusive antibody response in some sera. The use of genotype-specific P16-P25 test, which differentiates the infecting viruses according to antibody reactivity to either MVV-like or CAEV-like antigens (Lacerenza et al. 2006), indicated that the majority of the SRLVs circulating among sheep and goat populations in Jordan belongs to the MVV-like genotype. The percentage of positive sera detected with the SU5 peptide antigens was lower with respect to ELITEST and P16-P25, as a consequence of the high variability of the SRLV envelope proteins. The SU5 ELISA confirmed that MVV-like genotype was more represented among the small ruminant population in Jordan.

In conclusion, this study suggests that a combination of serological assays based on antigens of different nature and origin might improve the identification of small ruminants infected with SRLVs whose genetic and antigenic makeup is not yet characterized. Alternatively, genetic analysis of the strains circulating in the area of interest will allow the development of assays based on genotype-specific antigens.

Genetic characterization of Jordanian SRLV strains would help the set up of specific diagnostic tests and it would also provide phylogenetic information on the SRLVs circulating in Middle East, the Fertile Crescent region where domestication started.

Conflict of interest

None of the authors of this paper has a financial or personal relationship with other people or organizations that could
inappropriately influence or bias the content of the paper.

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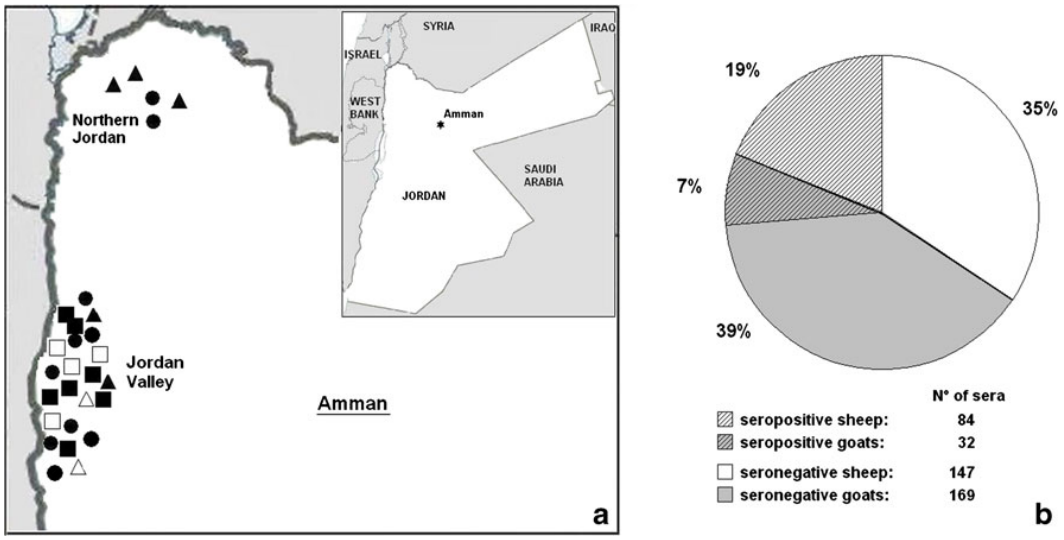

Fig. 1 Jordan map indicating flock distribution and sheep and goat seroprevalence. a Map showing the distribution of the flocks sampled in Northern Jordan and Jordan Valley: (black circle/white circle, seropositive/negative sheep flock; black square/white square, seropositive/ negative goat flock; black triangle/white triangle, seropositive/negative mixed flock); b percentage of positive and negative sera. Total number of sera analyzed, 432

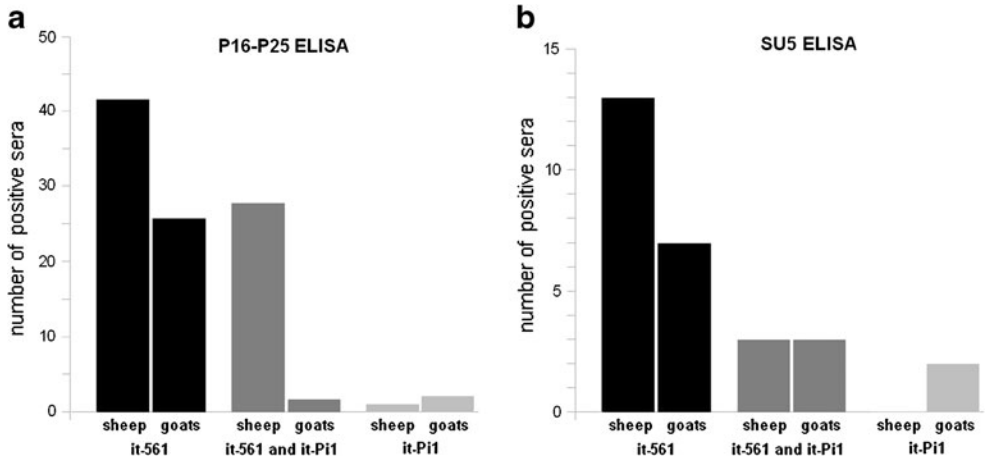

Fig. 2 Reactivity of sheep and goat sera to MVV-like It-561 and CAEV-like It-Pi1 antigens. a P16-P25 ELISA; b SU5 ELISA performed with individual antigens separately on sera reactive against pooled antigens. Black and light gray columns indicate the number of sera reacting exclusively to MVV-like and to CAEV-like antigens, respectively. The dark gray columns indicate the number of sera reacting to both MVV-like and CAEV-like antigens

Table 1 Response of sheep and goat sera to SRLV antigens

	Number of sera	ELITEST	P16-P25	ELITEST + P16-P25	Total
Sheep	231	13	16	55	84 (36.4 %)
Goat	203	2	28	2	32 (15.9)
Total	434	15	44	57	116

Sheep and goat sera reacting to ELITEST only, to P16-P25 only and to both tests. Within parentheses, sheep and goat seroprevalence