Journal of Animal and Veterinary Advances 9 (13): 1804-1810, 2010
ISSN: 1680-5593

Partial Characterization of Small Ruminant Lentiviruses from Goat from Sudan Based on GAG Gene Sequence Analysis

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Abstract: Caprine Arthritis Encephalitis Virus (CAEV) infection is widespread in the world; positive serology of the disease in Sudan was recently reported but genetic studies of the virus have not been carried out. In this study, a sequence of 354 nucleotides of the gag gene was presented covering part of the coding sequences for the Capsid (CA) p25 proteins for eight Sudanese isolates. Resulting nucleotide sequences were aligned along with those from other ovine/caprine prototypic and all Sudanese sequences appear to be closely related at both nucleotide and amino acid sequences to each other and to the prototypic CAEV-Co isolate. They were also found to be clearly divergent from ovine isolates which clustered in a separate group. Hypothesis as to how this virus was introduced into Sudan was discussed.

Key words: Caprine Arthritis Encephalitis Virus (CAEV), gag gene, nucleotide sequences, disease, amino acid, Sudan

INTRODUCTION

Caprine Arthritis Encephalitis Virus (CAEV) of goats and Maedi Visna Virus (MVV) of sheep belong to Small Ruminant Lentiviruses (SRLVs). Caprine arthritis encephalitis virus infection may lead to chronic disease of the joints and on rare occasion's encephalitis in goat kids <6 months of age. The disease was first diagnosed in goats in 1974 (Cork *et al*., 1974). Since that time, it has been diagnosed in goats in North America, Europe, Kenya, Peru, Australia, and New Zealand (Knoweles, 1997). CAEV is an RNA virus from the Lentivirus genus of the Retroviridae family (Cork *et al*., 1974). Their genomes have the typical proviral genomic organization of lentiviruses consisting of Long Terminal Repeats (LTR), the gag, pol and env genes open reading frames which encode for proteins with regulatory functions in viral replication (Clements and Zink, 1996). Gag and pol genes encode for core proteins and viral enzyme (i.e., reverse transcriptase), respectively and relatively conserved (Pepin *et al*., 1998). Env gene encodes for the surface and transmembrane glycoproteins and exhibits high heterogeneity (Pepin *et al*., 1998).

Complete nucleotide sequences of CAEV and MVV strains, i.e., CAEV-Co from United States (Saltarelli *et al*., 1990), K1514 from Iceland (Sonigo *et al*., 1985), SA-OMVV from South Africa (Querat *et al*., 1990) and EV-1 from Scotland (Sargan *et al*., 1991) in addition to some partial sequences have been published (Leroux *et al*., 1995; Chebloune *et al*., 1996).

SRLVs have previously been classified phylogenetically into at least six clades with no clear separation according to host species or geographical origin (Zanoni, 1998; Rolland *et al*., 2002). According to a recently proposed phylogentic organization based on gag and pol sequences (Shah *et al*., 2004), SRLVs can be subdivided genetically into groups A-D with subgroups present in groups A and B (Shah *et al*., 2004). Group A corresponds to the heterogenous MVV type and can further be subdivided into seven subtypes, designated A1-7. The second group (Group B) refers to the genetically less complex CAEV type and comprises only two distinct lineages termed subtypes B1 and 2.

Two additional SRLV Groups C and D have been recently identified on basis of their great genetic divergence with the two previous groups but are not yet fully described because they are either represented by few isolates or recognized based only on pol sequence (Shah *et al*., 2004). Sheep SRLVs prototypes such as South Africa SA-OMVV strain, the British EV-1 strain and

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Icelandic strain visna K1514 were all assigned to the A1 group which contained only sheep SRLV (Shah *et al*., 2004), goats prototypes such as CAEV-Co and CAEV-1GA (Gjerset *et al*., 2006) belonged to the B1 and C groups, respectively both containing only goat SRLV sequences. Groups A2 and D appeared to contain only sheep SRLV sequences, Groups A5 and 7 only contained goat SRLV sequences. However, groups A3, A4, A6 and B2 contained both sheep and goat SRLV sequences (Shah *et al*., 2004).

Infection with CAEV in goats from Sudan was recently reported (Elfahal *et al*., 2010). Infected goats develop arthritis and mainly exhibit no clinical signs. Infected goats were diagnosed by the presence of CAEV-specific antibodies and/or detection of CAEV-proviral DNA. In this study we describe the first sequence analysis of SRLVs of goats in Sudan. A fragment was sequenced from the gag gene covering partial part of the coding sequences for the Capsid (CA) P25 protein. Resulting sequences were aligned with those from other ovine/caprine lentiviruses isolates.

MATERIALS AND METHODS

Animals and blood samples: Eight goats from different sites in Khartoum State proven positive for CAEV infection by ELISA were analysed in this study. Whole blood (5 mL in EDTA vacutainer tubes) was collected from each of these animals.

DNA extraction: DNA was extracted according to Reina *et al*. (2006) using phenol-chloroform-isoamylic acid alcohol solution. In brief; Peripheral Blood Mononuclear Cells (PBMC) were separated on Ficoll-Paque (GE Healthcare, Sweden). PBMC were then lysed in buffer (100 mM Tris-HCl Ph 7.5; 12.5 mM EDTA-Na_2; 150 mM NaCl; 0.5% SDS) containing proteinase K at 55°C for 2 h or 37°C overnight. Genomic DNA was subsequently extracted twice with phenol-chloroform-isoamylic acid alcohol (25:24:1) and again extracted twice with chloroform-isoamylic acid alcohol (24:1). Following treatment with 96% ethanol and centrifugation, the precipitate was resuspended in distilled water (50 μL) and stored at -20°C till the day of the PCR testing.

PCR and sequencing: The PCR reaction was carried out using a Primus 96 thermal cycler (MWG-Biotech, Germany). Primers that target the CAEV gag gene of the CAEV-Co prototype strain (GenBank accession number M33677) were used to amplify 433 bp of the gag gene (Clavijio and Thorsen, 1996). DNA fragment were amplified in 25 μL reaction mixtures containing: 2.5 μL of 10×PCR buffer, 1.5 mM $MgCl_2$, 0.2 mM of each deoxynucleotide triphosphate (dNTPs), 400 nM of each primer, 2.5 U of Taq DNA polymerase (Promga, USA), 5% DMSO and 3 μL of DNA template.

The PCR amplification was achieved by 35 cycles each including a denaturation step at 94°C for 1 min, annealing step at 50°C for 30 sec and extension step at 72°C for 1 min. PCR amplified DNA fragments were analysed by electrophoresis on 1.5% agrose gels stained with ethidium bromide and visualized on a UV transilluminator.

PCR products were sent for commercial sequencing at Macrogen Company (Seoul, Republic of Korea).

Sequence analysis, GenBank accession numbers, alignment and phylogenetic tree: Nucleotide sequences obtained from sequencing the resulting 354-nucleotide fragments of the gag gene of the eight CAEV strains were submitted to GenBank and the accession numbers FJ619565-FJ619572 were assigned to them. Other SRLVs sequences used in this analysis were downloaded from GeneBank which include Cork-co, EV1, SA-OMVV and K1514 (Table 1). Further sequences were used in the tree to determine the group of the isolates (Table 1). The sequences obtained from the samples and their deduced amino acids were compared to sequences and amino acids in the GenBank database using the BLAST program and multiple sequences and amino acids alignment was generated using Clustal W (Thompson *et al*., 1994). Percentage divergence value-obtained from multiple sequence alignment were used to construct the phylogenetic tree.

For this purpose, nucleotide sequences were first aligned along with reference sequences obtained from GenBank using ClustalW program at DDBJ website. Thereafter, a phylogenetic tree was constructed using the Neighbor-Joining (NJ) method (Saitou and Nei, 1987) and genetic distances were calculated with the Kimura 2 parameter model. Phylogenetic tree was constructed using the nucleotide sequences and was performed by using DDJB website. Phylogenetic tree was finally drawn with Treeview software (Page, 1996).

RESULTS

Sequeces comparisons: The PCR products from the gag gene obtained from eight seropositive goats were sequenced (Table 1) and the sequences were submitted to the GenBank. Because a direct PCR product sequencing was carried out, an average number of 354 nucleotides out of the 433 nucleotides were obtained. These were translated and their 117 derived amino acids sequences

Table 1: SRLVs nucleotide sequences used in the study

Virus strain	Country	Accession No.	Species	Gene	Reference
CAEV-Co	USA	M33677	Goat	Whole	Saltarelli *et al.* (1990)
1GA	Norway	AF322109	Goat	Whole	Gjerset *et al.* (2006)
SA-OMVV	South Africa	M31646	Sheep	Whole	Querat *et al.* (1990)
EV1	Scotland	S51392	Sheep	Whole	Sargan *et al.* (1991)
K1514	Iceland	M60609	Sheep	Whole	Sonigo *et al.* (1985)
5526	Switzerland	AY454174	Goat	gag	Shah *et al.* (2004)
5776	Switzerland	AY454224	Goat	gag	Shah *et al.* (2004)
5723	Switzerland	AY454220	Goat	gag	Shah *et al.* (2004)
5717	Switzerlan	AY454217	Goat	gag	Shah *et al.* (2004)
5705	Switzerlan	AY454215	Goat	gag	Shah *et al.* (2004)
SUD01	Sudan	FJ619565	Goat	gag	This study
SUD02	Sudan	FJ619566	Goat	gag	This study
SUD03	Sudan	FJ619567	Goat	gag	This study
SUD04	Sudan	FJ619568	Goat	gag	This study
SUD05	Sudan	FJ619569	Goat	gag	This study
SUD06	Sudan	FJ619570	Goat	gag	This study
SUD07	Sudan	FJ619571	Goat	gag	This study
SUD08	Sudan	FJ619572	Goat	gag	This study

Table 2: Nucleotide and amino acid sequence diversity of Sudanese SRLV strains

Samples	SUD02	SUD03	SUD04	SUD05	SUD06	SUD07	SUD08
SUD01	0.86	0.86	0.57	0.86	1.10	0.29	3.1
	0.00	1.70	0.00	0.86	1.70	0.00	4.2
SUD02	-	1.70	1.40	1.70	2.00	1.10	3.4
	-	1.70	0.00	0.86	1.70	0.00	4.3
SUD03	-	-	1.40	0.57	0.86	1.10	2.8
	-	-	1.70	0.86	1.70	1.70	4.3
SUD04	-	-	-	1.40	1.70	0.86	3.1
	-	-	-	0.86	1.70	0.00	4.3
SUD05	-	-	-	-	0.86	1.10	2.8
	-	-	-	-	0.86	0.86	3.4
SUD06	-	-	-	-	-	1.40	3.1
	-	-	-	-	-	1.70	4.3
SUD07	-	-	-	-	-	-	3.1
	-	-	-	-	-	-	4.3

Percentage divergence values were obtained by comparisons of nucleotide sequences (plain number) and deduced amino acid sequences (number in bold) between the Sudanese strains

were aligned using Clustal W software along with the sequences of well-known prototypic strains of CAEV and MVV. The percentage of nucleotide and amino acid substitution were determined by pairwise comparisons between the eight Sudanese isolates (Table 2).

Interestingly, all sequences were found to be closely related to each other at both nucleotide and amino acid residues except for sample number 8 which displayed a lesser identity number to the rest of the samples but still closer to them, than to strains from other geographical areas. Divergences among the Sudanese samples range from 0.29-2% at the nucleotide level and from 0-1.7% at amino acid level (Table 2). Sample number 8 differed from the rest of the sequences in that it showed divergences from 2.8-3.4% at nucleotide level and 3.4-4.3% at the amino acid level.

Comparison with known Ovine/Caprine SRLV strains suggested that Sudanese isolates are divergent from the ovine strains, closely related to the CAEV-Co and revealed the following differences at the nucleotide level: 26% with SA-OMVV, 27.4% with K1514, 27.4% with EV1. Likewise at amino acid level, the average pairwise divergences were 6.1% with SA-OMVV, 6% with K1514, 8.8% with EV1 ovine strains. On the other hand, comparison with CAEV-Co caprine prototypic strain revealed an average 0.95 and 1.1% differences at nucleotide and amino acid sequence, respectively (Table 3).

Sequence alignment was performed to clarify the position of differences at nucleotide and amino acid sequences between the Sudanese isolates and some known isolates of Ovine/Caprine SRLV. In these alignments, 354 residues of the sequence were used and the CAEV-Co was chosen as a reference sequence. The alignment has shown an overall identity of 60.45 and 66.67% at nucleotide and amino acid sequences respectively (Fig. 1 and 2) and revealed a total of 27 polymorphism sites (Fig. 1).

All sequences from Sudan were found to be closely related to each other and to the CAEV-Co strain and showed more divergence from the sheep isolates at nucleotide and amino acid level (Fig. 1 and 2). The alignment displayed three gaps at positions 324, 338, 344 of the sequenced product (Fig. 1).

Table 3: Nucleotide and amino acid sequence diversity between Sudanese and other SRLV strains

Virus strain	SUD01	SUD02	SUD03	SUD04	SUD05	SUD06	SUD07	SUD08
CAEV-Co	0.57	1.40	0.29	1.10	0.29	0.57	0.86	2.5
	0.86	0.86	0.86	0.86	0.00	0.86	0.86	3.4
SA-OMVV	25.60	25.40	26.00	25.60	25.70	26.30	25.90	27.5
	6.00	6.00	6.00	6.00	5.20	6.00	6.00	7.7
K1514	27.40	26.70	26.70	27.70	27.40	28.00	27.10	28.0
	6.00	6.00	6.00	6.00	5.20	6.00	6.00	7.6
EV1	27.30	27.00	27.60	27.60	27.40	27.90	27.00	27.0
	8.60	8.60	8.60	8.60	7.80	8.60	8.60	11.1

Percentage diversity values were obtained by comparisons of nucleotide sequences (plain numbers) and deduced amino acid sequences (number in bold) between Sudanese and known SRLV strains (GenBank accession numbers are in Table 1)

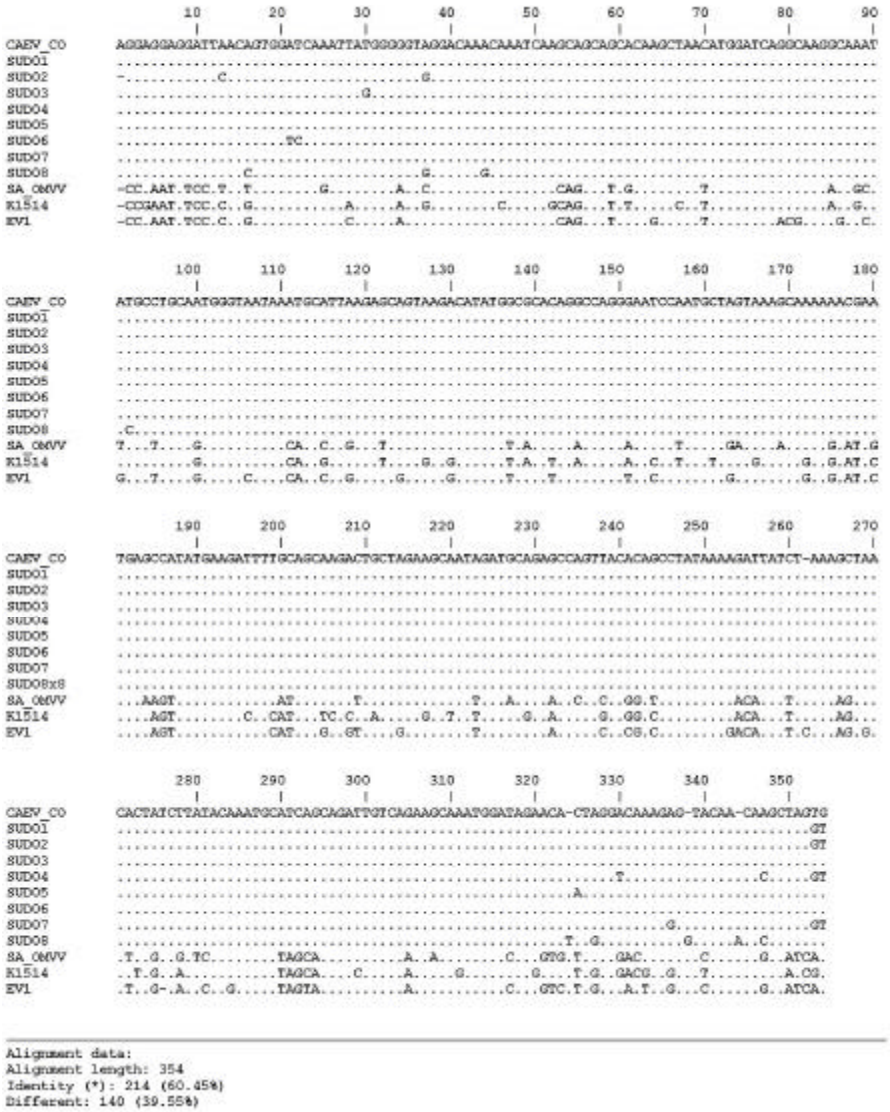

Fig. 1: Nucleotide sequence alignment of the gag gene from eight Sudanese isolates of CAEV with the sequences of prototype CAEV and ovine prototypes. Only nucleotides differing from the CAEV-Co sequence are shown. Dots indicate identity with CAEV-Co whereas gaps are represented by dashes

Phylogenetic analysis: Phylogenetic analysis of the Sudanese isolates compared to those from known prototypic strains of ovine/caprine lentiviruses was performed using the neighbor-joining method. The

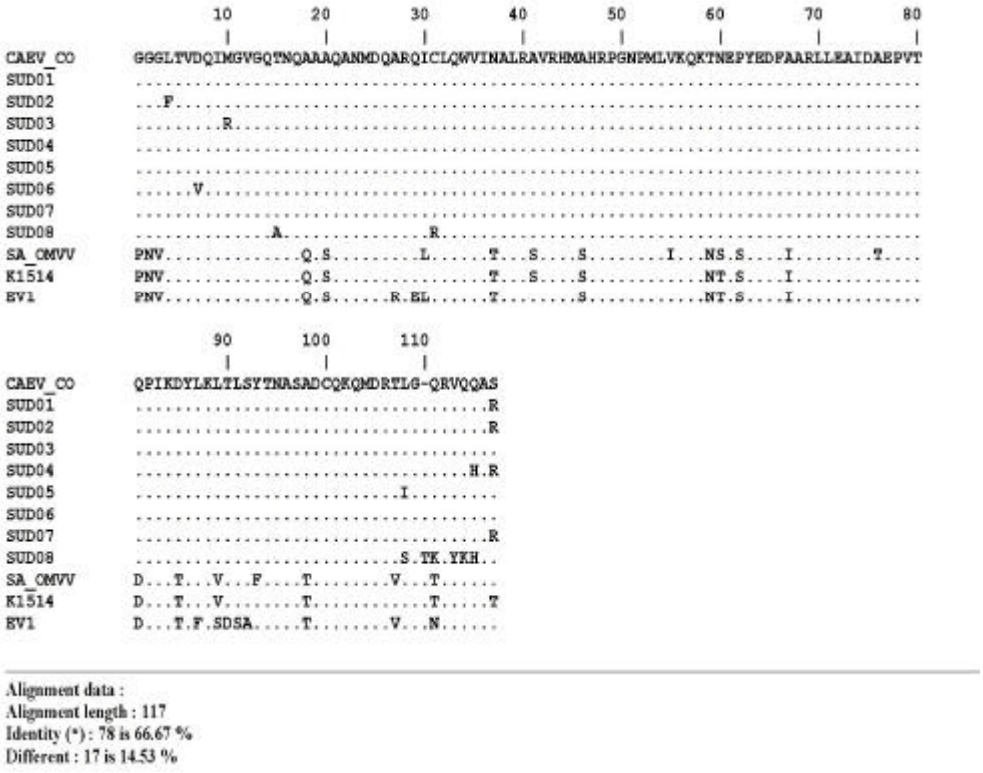

Fig. 2: Alignment of amino acid sequences from the gag gene encoding capsid protein. Only amino acids differing from the CAEV-Co sequences are shown. Dots indicate identity with CAEV-Co, whereas gaps represented by dashes

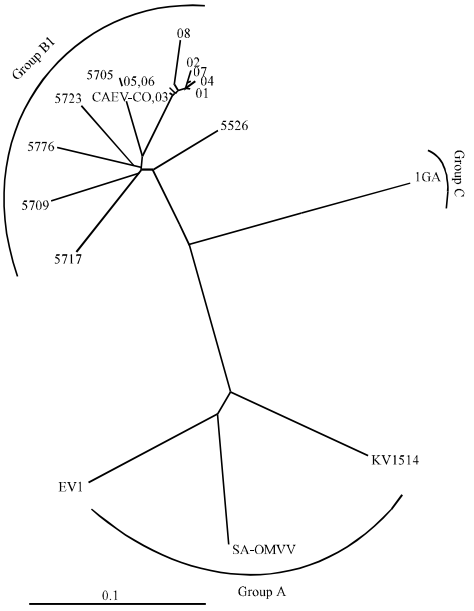

Fig. 3: Phylogenetic tree obtained using neighbour joining method with the Kimura program showing the relationship between Sudanese isolates (No. 01-08) and other known SRLV strains

additional sequences used were some isolates from the GenBank that belong to Group B1 (Shah *et al*., 2004) and the Norwegian isolate that belong to group C (Gjerset *et al*., 2006) (Table 1). The derived phylogenetic tree is shown in Fig. 3. The analysis revealed that Sudanese isolates were closely related to the CAEV-Co, both clustering in one group that also contained sequences of Group B1 of Shah *et al*. (2004). This result confirmed that the Sudanese sequences actually fall within group B subgroup B1 which contain isolates from goats only (Shah *et al*., 2004).

The ovine strains K1514, EV1, SA-OMVV were in a separate group (Group A) and the Norwegian isolate in a third separate group (Group C) (Fig. 3).

DISCUSSION

Small Ruminant Lentiviruses (SRLVs) infections including Caprine Arthritis Encephalitis Virus (CAEV) and Maedi-Visna Virus (MVV) are widespread in many countries all over the world and they cause substantial economic damage. SRLV infections persist for life and carriers are considered a continuous potential source of virus for transmission. A few complete genomes and

many partial sequences of SRLV strains have been isolated and characterized in many European countries, South Africa and in USA (Angelopoulou *et al*., 2005). In the Sudan SRLV strains have never been isolated or characterized.

In the present study, we described the analysis of 354 nucleotide residues and their deduced 117amino acids of gag gene of SRLV strains from 8 goats in Khartoum State. The sequences of gag gene for the comparison and phylogenetic analysis was focused because this region is well known to be specific for the group of lentiviruses and have less divergences compared to those of the envelope gene (Rolland *et al*., 2002).

The data indicated that the isolates (except for SUD08) which have been collected from different areas in Khartoum state are very similar and closely related to each other differing by an average of 1.1% at the nucleotide sequence and by 0.97% at the amino acid sequence. This is in line with Kuzmak *et al.* (2007) who reported that isolates which were extracted from Polish goats were found to be closely related to each other and to the CAEV-Co based on gag gene. One isolate, SUD08, differed more from all other Sudanese samples and showed differences of 3.1% at nucleotide sequence and 4.2% at the amino acid sequence but still closely related to them. The nucleotide sequences in the gag region of the Sudanese isolates appeared to be more similar to the CAE-Co caprine prototypic (0.95% divergence) than to the ovine prototypic strains (27% divergence) at nucleotide sequence as we further confirmed by the phylogenetic tree.

Phylogenetic analysis showed that Sudanese isolates lied within the CAEV-Co group and closely related to this prototypic isolate. All of the isolates lied among the isolates introduced from the GenBank and this confirmed that sequences are in the group B in subgroup B1 which contained isolates from goats only and this is in line with Shah *et al*. (2004).

CONCLUSION

Sudanese isolates investigated here in this study are related to each other, similar to the CAEV-Co prototypic and clustered in group B1. The isolates are more similar to the CAEV-Co than to Shah Isolates of the same group and most divergent from sheep isolates.

The study showed that CAE viruses infecting goats in Sudan might have arisen from the same source as the isolates nucleotide and amino acid sequences were closely related to one another and related to those isolated from goats in the other parts of the world.

ACKNOWLEDGEMENTS

We are grateful to Prof. Stephen Valas (National Reference Laboratory for Small-Ruminant Lentiviruses, France) for kindly providing DNA positive control.

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