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First Report of Caprine Arthritis Encephalitis Virus Infection in Sudan

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Abstract: Caprine Arthritis-Encephalitis Virus (CAEV) infection of goats has a worldwide distribution and trade in live animals is considered the main reason for the widespread of the disease. The disease has not been previously recognized in the Sudan neither by serological nor molecular biological methods. The objective of this study was to investigate CAEV infection among the imported purebred goats and their crossbred lines in the Sudan, generate information about the disease and to establish methods for diagnosis of CAEV infections, as a base line for further epidemiological and virological studies. In this study, samples (273 serum and 173 whole blood) were collected from goats in different areas of Khartoum state, Sudan. Enzyme Linked Immunosorbant Assay (ELISA) was used to detect CAEV-specific antibodies in serum and Polymerase Chain Reaction assay (PCR) was performed to detect CAEV nucleic acid in blood samples. Out of 273 animals tested, 20 (7.3%) were confirmed as CAEV infected by ELISA and nucleic acid of CAEV was detected in 41 (23.7%) out of 173 animals by PCR. ELISA failed to detect 24 samples that were positive by PCR, while PCR failed to detect only two samples that were positive by ELISA. In the present study, we reported for the first time the existence of CAEV infection, using ELISA and PCR in goats in Sudan.

Key words: CAEV, small ruminant, lentiviruses, PCR, MUV, Sudan

INTRODUCTION

Caprine Arthritis-Encephalitis Virus (CAEV) and Maedi-Visna Virus (MVV) or Ovine Progressive Pneumonia Virus (OPPV) are closely related Small Ruminants Lentiviruses (SRLVs) that cause persistent virus infections in goats and sheep respectively.

Caprine arthritis-encephalitis virus may lead to chronic disease of the joints and on rare occasions encephalitis in goat kids less than six months of age. The disease was first diagnosed in goats in 1974 (Cork *et al*., 1974 a, b). 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 a, b). 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 the relatively conserved core proteins and viral enzyme (i.e., reverse transcriptase), respectively (Pepin *et al*., 1998), env gene encodes for the surface and transmembrane glycoproteins and exhibits high heterogeneity (Pépin *et al*., 1998). The major mode of transmission of the CAEV is via colostrum or milk (Adams *et al*., 1983). Other potential sources of viral transmission include breeding of an infected animal with non-infected one, transmission in utero, via saliva or respiratory secretion, contact with infected blood and viral contamination of milking equipments, needles and tattooing equipment (Adams *et al*., 1983).

Diagnosis of the CAEV is made by serological methods including Enzyme Linked Immunosorbent Assay (ELISA) and Agar Gel Immunodiffusion (AGID) test but these may not detect the virus infection in case of animals with low levels of antibodies that may also take several months to develop (Houwers and Nauta, 1989). Diagnosis by virus isolation on susceptible cell cultures, despite its sensitivity is both time-consuming and expensive therefore, it is not advisable for routine diagnostic purpose.

These diagnostic problems can be overcome by the use of Polymerase Chain Reaction (PCR), which is specific, rapid and sensitive. Recently, various PCR methods for detection and diagnosis of CAEV were developed (Reddy *et al*., 1993; Barlough *et al*., 1994; Zanoni *et al*., 1996; Leroux *et al*., 1997; Wagter *et al*., 1998). Meager information is available regarding SRLV infection in Sudan. Osheik *et al*. (1995) using AGID failed to detect CAEV and MVV specific antibodies in local

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Sudanese goats and sheep. Of recently, several cases of chronic arthritis were noticed in pure (Saanen) foreign breed and crossbred goats in several areas of Khartoum State.

The present study aimed to investigate CAEV infection among goats, particularly the imported Saanen breed and their crosses, which existed in Sudan for genetic improvement programs.

MATERIALS AND METHODS

Animals and blood samples: Two hundred and seventy three (pure Saanen and their crossbred lines) goats farms with genetic improvement programs at various locations in Khartoum State were sampled in this study. Samples included 273 whole blood samples for serum (5 mL blood without EDTA) and 173 whole blood samples (5 mL with EDTA) for nucleic acid isolation. Serum was separated from the blood and stored at -20°C until used. Peripheral Blood Mononuclear Cells (PBMC) were isolated from the 5 mL EDTA-blood on ficoll-paque (GE Healthcare, Sweden) and stored at -20°C until DNA extraction.

ELISA: ELISA test was performed using competitive ELISA kit (VMRD; United Kingdom) according to the manufacturer instructions. These kits were used to detect antibodies against the CAEV envelope glycoprotein (gp135).

DNA extraction: DNA was extracted from PBMC according to Reina *et al*. (2006) using phenol-chloroform method. PBMC were lysed in 800 μL of lysis buffer (100 mM tris-HCl pH 7.5; 12.5 mM EDTA-Na2; 150 mM NaCl; 0.5% SDS) then 10 μL of proteinase K (Promega, USA) were added and the samples were incubated at 55°C for 2 h or at 37°C overnight. Genomic DNA was subsequently extracted twice with phenol-chloroform isoamylic acid alcohol (25:24:1) and then extracted twice with chloroform-isoamylic acid alcohol (24:1). Following treatment with absolute ethanol and centrifugation, the precipitate was resuspended in distilled water and stored at -20°C till used for PCR.

Gag gene-PCR: Primers used to amplify the group associated antigen (gag) gene were those described by Clavijio and Thorsen (1996), their sequence as follows:

Forward: 5′AGGAGGAGGATTAACAGTGG-3′

Reverse: 5′TCCTGGCCTTAATGCTTGTG-3′

The length of the expected amplification fragment was 433 pb for gag gene region. Primers purchased freeze dried from Alpha DNA (Montreal, Quebec, Canada) were reconstituted with 1 mL DDW and left overnight at 4°C to completely dissolve. Working solutions of each primer were prepared so as to contain 10 pmol μL^{-1}.

PCR amplification: The PCR reaction was carried out in primus 96 thermal cycler (Germany). DNA fragment were amplified in 25 μL reaction mixtures containing approximately 3 μL of genomic DNA, 1.5 mM $MgCl_2$, 0.2 mM each Deoxynucleoside Triphosphates (dNTPs), 400 nM of each primer and 2.5 U Taq DNA polymerase (promega, USA) and 5% DMSO.

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% agarose gels stained with ethidium bromide and visualized on a UV transilluminator.

RESULTS

Two hundred and seventy three serum and 173 DNA samples from different locations in Khartoum State, Sudan were analysed for the presence of anti-CAEV antibodies using ELISA and for CAEV proviral DNA using PCR assay, respectively. Serology revealed 20 out of 273 samples were positive by ELISA giving an overall prevalence of 7.3% and prevalence of 8.6, 17.6 and 1.4% were recorded for Khartoum North, Omdurman and Khartoum areas, respectively (Table 1). Regarding the breeds of the sampled goats, 6 (4.5%) out of 133 pure Saanen and 14 (10%) out of 140 crossbred goats were found positive (Table 2). When PCR products were analyzed by agarose gel electrophoresis and ethidium bromide staining, a total of 41 (23.7%) out of 173 goats had a single amplification band of the expected (433 bp) size (Fig. 1). The distribution of PCR positive animals according to the area was as follows: in Khartoum North 26 (22.2%) out of 117, in Omdurman 7 (41.2%) out of 17 and in Khartoum 8 (20.5%) out of 39 (Table 3). Regarding breed, 20 (19.6%) out of 102 pure Saanen and 21 (29.6%) out of 71 cross bred goats were positive by PCR (Table 4).

Table 1: Seropositivity in goats from 3 locations in Khartoum State

Locations	Sample	No. +ve	+ve (%)
Khartoum North	185	16	8.6
Omdurman	17	3	17.6
Khartoum	71	1	1.4
Total	273	20	7.3

Table 2: Seropositivity in goat breeds as detected by ELISA

Breed	Sample	No. +ve	+ve (%)
Saanen	133	6	4.5
Crossbreed	140	14	10
Total	273	20	7.3

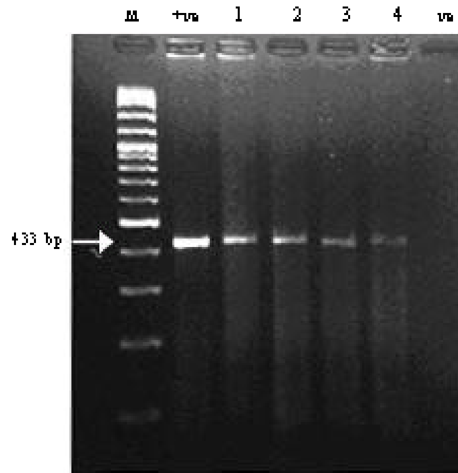

Fig. 1: PCR amplification of the Caprine Arthritis Encephalitis Virus (CAEV) gag gene. Sample PCR products in 1.5% agrose gel showing an amplified target of 433 bp from the gag gene. Lane M, DNA molecular marker. Lane +ve, positive control. Lanes 1, 2, 3 and 4, positive samples showing expected band. Lane -ve, Negative control

Table 3: Result of gag-PCR of goats from different locations in Khartoum State

Locations	Sample	No. +ve	+ve (%)
Khartoum North	117	26	22.2
Omdurman	17	7	41.2
Khartoum	39	8	20.5
Total	173	41	23.7

Table 4: Result of gag-PCR among goats breeds

Breed	Sample	No. +ve	+ve (%)
Saanen	102	20	19.6
Crossbreed	71	21	29.6
Total	173	41	23.7

Table 5: Comparison the results obtained in 173 tested using ELISA and PCR techniques

Techniques	PCR (+)	PCR (-)	Total	Concordance (%)
ELISA (+)	17	2	19	
ELISA (-)	24	130	154	85
Total	41	132	173	

We used the 173 samples that were tested using the two techniques to compare the results between the ELISA and PCR. Of these 41 samples were found positive by PCR, 17 were positive by both ELISA and PCR, 2 were positive by ELISA only and 134 were negative by both methods. All PCR negative goats were found also negative by ELISA except for two goats, which were negative by PCR but positive by ELISA (Table 5). This revealed an overall agreement of 85% (147/173) between the two tests (Table 5).

DISCUSSION

Small ruminant lentiviruses 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. SRLVs infections persist for life and carriers are considered a continuous potential source of virus infection. In Africa, surveys indicated evidence of SRLVs in Kenya (Wandera, 1970), Morocco (Mahin *et al*., 1984), Nigeria (Belino and Ezeifeka, 1984), Mozambique (Pereira *et al*., 1989) and Algeria (De Boer *et al*., 1979). These countries imported animals from Europe and this facilitated the introduction of the disease and substantiated the fact that spread of SRLVs occurred via importation of animals (De Boer *et al*., 1979; Adams *et al*., 1980). Furthermore, Konishi *et al*. (2004) in Japan and Torres-Acosta *et al*. (2003) in Mexico reported evidence of CAEV in goats which were imported for genomic improvement programs. These researchers indicated that the CAEV was most prevalent in imported goats (Torres-Acosta *et al*., 2003) and was non-existent in goats not in contact with imported goats, while it was more common in animals in contact with imported goats.

Recently in the Sudan, the concerned veterinary authorities started regulating imports of live goats for genetic improvement allowing only the introduction of animals that were bought from goat flocks certified free of the disease. However, animals that carry the virus may not show any clinical signs. Serological latency may also take months or years before seroconversion takes place or may never occur at all, which is a well known feature of SRLVs (Houwers and van der Molen, 1987; Houwers and Nauta, 1989; Johnson *et al*., 1992; Rimstad *et al*., 1993; Wagter *et al*., 1998; Eltahir *et al*., 2006). In addition, CAEV infected nimals may also fail to develop antibodies levels detectable by serological tests (Krassnig and Schuller, 1998; Eltahir *et al*., 2006). There is only meagre information available about SRLV infection in the Sudan. A preliminary survey by Osheik *et al*. (1995) indicated the absence of antibodies to SRLVs in Sudanese goats and sheep by AGID test. In this study, we used competitive ELISA (cELISA) based on gp 135 recombinant antigen to detect antibodies of CAEV. The advantage of the use of cELISA is that undiluted serum could be used, which allows detection of sera with low titers of antibodies and minimizes the prevalence of false negative (Herrman *et al*., 2003). In the present study, 273 animals from different areas of Khartoum state were tested for CAEV antibodies using ELISA and 173 animals for SRLV proviral DNA using PCR. The results revealed that 20 (7.3%) samples were positive in ELISA and 41 (23.7%) were positive by PCR. Infections were detected in all localities examined in Khartoum State and positive goats were detected in all

areas by ELISA including Khartoum North (8.6%), Omdurman (17.6%) and Khartoum (1.4%) indicating the widespread nature of infection in this state. PCR amplification had been described for detection of CAEV DNA in cultured cell using primers for the pol gene (Zanoni *et al*., 1990) and in PBMC and milk from infected goats using primers for the gag gene (Reddy *et al*., 1993; Rimstad *et al*., 1993; Clavijio and Thorsen, 1996). DNA from PBMCs was used as templates, with reported sensitivities ranging from 70-95% (Reddy *et al*., 1993; Travassos *et al*., 1999; Wagter *et al*., 1998; Extramiana *et al*., 2002; Eltahir *et al*., 2006; Kuzmak *et al*., 2007).

The PCR assay using DNA from PBMC as target in the present study showed an overall prevalence of (23.7%). This is in agreement with Karanikolaou *et al*. (2005), who found a similar result (23.8%) in Greece. PCR also detected positive goats in all areas including Khartoum North (22.2%), Omdurman (41.2%) and Khartoum (20.5%).

In the results, all PCR negative goats were found also negative for viral antibodies except for two goats, which were negative by PCR and seropositive by ELISA. This is in agreement with Kuzmak *et al*. (2007), who reported 33% of seropositive goats as negative in PCR. This could be attributed to the low number of infected monocytes in the blood or to the low viral load in the post-seroconversion phase (Karanikolaou *et al*., 2005; De Andres *et al*., 2005; Eltahir *et al*., 2006). In this study, PCR detected 41 samples as positive, 17 of which were positive by ELISA, while 24 samples were positive by PCR and negative by ELISA. This result indicates that PCR may detect infected animals prior to seroconversion. This is supported by Wagter *et al*. (1998), who reported that out of 9 seronegative goats that were PCR positive, six seroconverted at later periods and Rimstad *et al*. (1993) detected 20 seronegative goats as PCR positive of which 10 seroconverted at later time. This indicates that SRLV variants could be detected by PCR in the absence of detectable antibody level. In the present investigation, the higher prevalence of CAEV infection in goats detected by ELISA and PCR in Omdurman locality could be attributed to the small number of the samples collected. Finally, this result further confirmed that PCR is a sensitive method, capable of detecting infected animals prior to seroconversion and that the combination of serology and PCR might be optimal for detection of infected animals especially for the control of the disease.

CONCLUSION

In the present study, we recorded the first detection of CAEV in Sudan using ELISA and PCR in blood samples from goats in Khartoum State. The information obtained in this study is considered an important first step towards the establishment of further control on imported animals for genetic improvement. This study showed that CAEV has been introduced into Sudan and is spreading as evidenced by the infection of the crossbred animals. This may indicate that cross breeding using infected animals is the main setting for spreading the disease in the Sudan. We have used a PCR assay that is simple and highly sensitive but still amenable to further improvement. We consider that this PCR can be an excellent tool to elucidate fully the epidemiological picture of SRLVs infections in Sudan.

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