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The First Molecular Detection of Caprine Arthritis Encephalitis Virus (Caev) in Iran

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Abstract

Caprine arthritis encephalitis virus (CAEV) is a retrovirus that naturally infects goats and induces chronic inflammatory and degenerative disease. Infection of goats has a worldwide distribution but it has not been previously recognized by molecular method in Iran. For this reason, 95 blood samples were taken from 4 months to 3 years goats to determine naturally infected animals with caprine arthritis-encephalitis virus (CAEV) by using polymerase chain reaction (PCR) method. The PCR primers were designed to amplify a 433 base pair region of the proviral gag gene. Results showed that 15.7 percent of sampled goats (15 cases) were positive for CAEV. The present study was confirmed the presence of CAEV genome in goats flock of Iran for the first time.

Keywords: Goat; Arthritis; Encephalitis; PCR; Gag Gene

Introduction

Caprine arthritis-encephalitis virus (CAEV) belongs to the lentivirus subfamily of retroviruses [1] which causes chronic progressive arthritis, pneumonia, and mastitis in adult goats and leukoencephalomyelitis in young kids [2,3]. Disease is widespread in populations of domestic sheep and goats throughout the world [4] and its high prevalence especially in industrialized countries (80-95% in the breeding stocks) is a major concern [5,6]. Ingestion of virus-infected colostrums or goat milk [7] is the main way of disease transmission and other routes such as direct contact, bodily secretions, and excretions [8,9] are less abundant. Prevalence of CAE around the world has passed over a wide range of variation among different countries, which may be as low as 1.9% in Turkey and 3.6% in Mexico, or even up to as high as 73% in USA and 82% in Australia [10]. Recent study from Iran showed that the 10.87 % of goats were serologically infected for CAEV [11] but unfortunately there is no previous data about the prevalence of this disease by molecular methods in Iran.

To our knowledge, most infected goats are asymptomatic and so far there is no effective treatment and supportive drugs are usually expensive. On the others hand, previous study suggested the high reduced production and significant economic losses due to disease [12]. Therefore, using a rapid, fast and sensitive method is essential for its diagnosis. Recent studies showed the high sensitivity, specificity and accuracy of molecular methods like Polymerase Chain Reaction (PCR) for rapid detection of CAEV in clinical samples [13,14]. Therefore, this current study was carried out for detection of CAEV in slaughtered goats by using PCR method in Iran.

Material and Methods

Animals

Ninety five slaughtered goats were sampled during the experiment. Goats were a member of different flocks and were randomly chosen among slaughter. Flocks were located in different geographic areas of the Chahar Mahal va Bakhtiyari province of Iran.

Samples

After giving an identification number, blood samples were taken and age, sex, and origin of animals were recorded. Additionally, based on dentition and owner's information, the age was determined. Blood samples were taken from jugular vein and mononuclear cells (PBMC) were isolated from 10 ml EDTA-blood on a Ficoll-Hypaque gradient. They were then destroyed in buffer (100 mM Tris–HCl pH 7.5; 12.5mM EDTA-Na_2; 150 mM NaCl; 0.5% SDS) containing Proteinase K (Sigma) (50 µg per 10^7 PBMC) at 55° for 2 h. Genomic DNA was subsequently extracted twice with phenol–chloroform–isoamylic acid alcohol (25:24:1, v/v/v, Sigma). It was then again extracted twice with chloroform–isoamylic acid alcohol (24:1, v/v, Sigma). Following treatment with 96% ethanol (Sigma) and centrifugation, the precipitate was re-suspended in distilled water (40 µl) and stored at 4°C till the day of the PCR test [15].

Gag gene-PCR

Primers used to amplify the group associated antigen (gag) gene were those described by Clavijo and Thorsen [16], their sequence as follows:

Forward: 5' AGGAGGAGGATTAACAGTGG-3'

Reverse: 5' TCCTGGCCTTAATGCTTGTG-3'

PCR amplification

The PCR reaction was carried out in primus 96 thermal cycler. 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 2.5U Taq DNA polymerase 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 analyzed by electrophoresis on 1.5% agarose gels stained with ethidium bromide and visualized on a UV trans illuminator [15].

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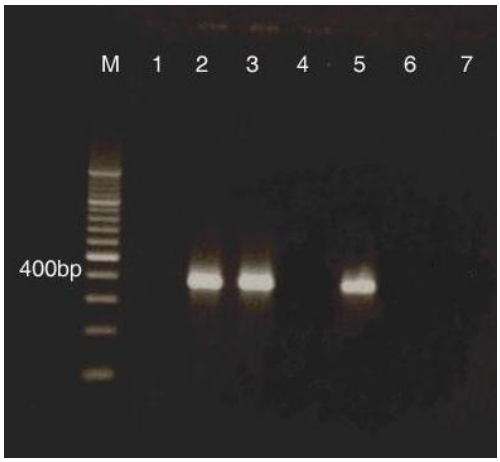

Figure 1: Agarose gel electrophoresis and ethidium bromide staining. Lane M indicates standard DNA marker; 1, negative control; 2, positive control; lanes 3 and 5 positive samples; lanes 4, 6 and 7 negative samples; the 433-bp PCR product has also been indicated.

Results

A total of 95 whole blood samples were taken from apparent healthy goats between June and August 2013. The goats were of various mixed breeds, and their ages ranged from 4 months to 3 years. Peripheral blood mononuclear cells (PBMC) were separated using the Ficoll gradient centrifugation method and total DNA was extracted from PBMC for confirmation by polymerase chain reaction. PCR products were analyzed by gel electrophoresis and ethidium bromide staining. A total of 15 out of 95 crossbred goats had a single amplification band of the expected (433 bp) size (Figure1). It is also showed that there was no significant difference between male and female goats PCR results using Q-square test (P>0.05). Based on these findings, infection rate among Shahrekord district goats were 15.7%. From 95 samples, 6 of 35 (17.14%) and 9 of 60 (15%) samples that were taken from males and females, respectively, were positive.

Discussion

Our study showed that the PCR method is good compromise for CAEV detection. PCR amplification had been described for detection of CAEV DNA in cultured cell using primers for the pol gene [17] and in PBMC and milk from infected goats using primer for the gag gene [16,18,19,]. DNA from PBMCs has used as templates, with reported sensitivities ranging from 70-95% [14,18]. The PCR assay in the present study showed an overall prevalence of (15.7%). This is in agreement with Karanikolaou et al. [20]. For the first time, our finding has confirmed the presence of CAEV genome in PBMC of apparently healthy Iranian goats.

CAEV is a lentivirus that persistently infects goats and sheep. The finding that CAEV and Maedi-Visna viruses frequently cross the species barrier between goats and sheep, and vice versa, has changed our view of the epidemiology of these viruses that are now referred to as small ruminant lentiviruses (SRLV) [15]. CAEV is transmitted from infected mothers to their offspring, mainly via ingestion of infected colostrum and milk. CAEV induces overt pathology in about one third of the infected animals. The frequency of affected animals varies in different goat families, pointing to an important genetic component in this disease. The principal manifestations are encephalitis and interstitial pneumonia in young animals, whereas arthritis and mastitis predominate in adult goats [21].

Our results showed that the prevalence of CAEV in goats in Iran was 15.7% which was higher than Mexico (3.6%) [22], Italy (4.0%) [23] and Great Britain (10.3%) [24] but entirely lower than USA (73%) [25] and Norway (86%) [26]. Serological study in Hungary showed that 30% of the Hungarian goat population was infected with CAEV [27]. Previous study from Thailand showed that the prevalence of CAEV was 12.4% [28]. Another study from Brazil showed that the total prevalence of CAEV in goats is 8.2% which was lower than our study. In total, the prevalence of CAEV around the world has passed over a wide range of variation which may be as low as 3.6% in Mexico and 15.7% in Iran (our study) until 82% in Australia [10]. Studies indicated that contact with goats from other herds, purchased animals into herd, presence of sheep in the farms and finally herd size are the main risk factors for epidemiology and prevalence of CAEV [29,30].

Comparison between the PCR and an antibody ELISA indicated very strong agreement between the two assays (kappa=0.912) and it was recognized that four of 7 goats with indeterminate ELISA results were PCR-positive [31]. Unfortunately, studies about the prevalence of CAEV by PCR method are very low throughout the world.

To our knowledge, our study is the first prevalence report of direct detection of CAEV from slaughtered goats by using PCR in Iran. Our results revealed the uses of molecular methods especially PCR for rapid and accurate detection of this virus in clinical samples. The results of this study showed that 17.14% of males and 15% of female goats were positive for presence of CAEV in Iran.

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