Iranian Journal of Virology 2023;17(1):63-69.

Original Article

Molecular Epidemiology of Caprine Arthritis Encephalitis Virus (CAEV) in Imported and Indigenous Dairy Goat Breeds in Iran

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Abstract

Background and Aims: Virus of caprine arthritis encephalitis belongs to the Retrovirdae family, which can cause arthritis, mastitis and abortion in adult goats, and encephalitis in kids especially in dairy breeds. Therfore, income of farmers are affected .since, in the recent years many importation of small ruminant animals especially dairy goats breeds to our country have been done . This study was conducted to evaluate infection of CAEV in imported and indigenous dairy goat breeds which were kept together in one herd.

Materials and methods: In this study, blood samples were taken from 249 dairy goats in one of the industrial breeding farm. Buffy coat was separated from the samples using centrifugation and DNA was extracted from them. Nested polymerase chain reaction (Nested PCR) method was performed with specific primers in order to detect the gag gene.

Results: The gag gene was detected in21 blood samples (8.3%) out of 249 samples and result of CAEV Infection rate was 11 (13.8%), 7 (8.1%) and 3 (3.6%) for Alpine, Saanen and Indigenous breeds, respectively. Percent of infection based on old was 15.8%, in < two years goats, 8% in 2 3 years and 7.7% > three years old.

Conclusion: Unfortunately, the imported and local breed of dairy goats were infected with CAEV, therefore, phylogeny studies are required to for identification of prevalence of the disease in herds of the country.

Keywords: Caprine arthritis encephalitis, CAEV, Nested PCR, gag, Iran

Introduction

Caprine encephalitis arthritis virus (CAEV) is classified as small ruminant lentiviruses (SRLV), this virus is one of the two lentiviruses belonging to the Retroviridae family currently known to infect sheep and goats (1, 2). Caprine arthritis-encephalitis (CAE) was first clinically recognized in Switzerland in 1959, with chronic arthritis in adult goats (3) and was first diagnosed in the United States in goats in 1974 (4). And since then it has been recognized in many countries in the world (5), Italy (6), Poland (7), Taiwan (8), Japan (9), South Korea (10), Australia (11), Iraq (12), Saudi Arabia (13), Sudan (14), Syria (15), Iran (16).

The SRLV genome consists of positive-sense RNA dimers of approximately 9 kb in size containing long terminal repeats (LTRs), gag (group-specific antigens), pol (polymerase), env (envelope) genes, and a number of regulatory genes. . The regulatory genes of gag and pol genes are well conserved among SRLVs,

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which makes them ideal targets for PCR primer design (17).

Body secretions and excretions are a potential source of virus transmission. The virus is transmitted through colostrum and milk, which is one of the main routes of CAEV transmission from mother to child and through vertical (2) or horizontal contact between infected and healthy adult animals (18). In addition, horizontal transmission through the respiratory tract after direct exposure to respiratory secretions or droplets has been reported among adult goats (2). Therefore, due to the possibility of horizontal and vertical transmission of CAEV, there is also a possibility of transmission of the disease by infected semen during mating and artificial insemination, which requires more detailed investigations (19, 20).

CAE virus causes subclinical disease because of its long incubation period and causes persistent lifelong chronic infection in the host. The most important clinical findings include progressive and intractable polyarthritis in adult animals and demyelinating encephalitis in goats younger than 2-6 months. Other clinical signs include mastitis, reported as the "hard udder" syndrome, which leads to decreased milk production, progressive paralysis, neurological dysfunction, chronic interstitial pneumonia, and excessive weight loss (18, 21).

SRLVs based on gag and gag-pol genes and LTR sequences include five genotypes (A-E) (22) genotypes A and B are MVV and CAEV strains, respectively, which were the most prevalent worldwide, while genotypes C, D and E were found only in Europe, namely, group C strains in Norwegian sheep and goats, genotype D strains in sheep and goats in Switzerland and Spain, and genotype E in Italy (23, 24).

The study of CAEV in Iran was limited to three previous studies in Khuzestan, Chaharmahal and Bakhtiari and Kerman provinces, which were carried out in recent years in the indigenous goat population of Iran. Considering that the main way of CAEV transmission is through milk, CAEV disease has not been investigated in dairy goats, especially breeds imported to Iran, and also considering the desire to keep these breeds, the study of this virus in imported dairy (Saanen, Alpine) and indigenous breeds is important, Therefore, this study was conducted with the aim of detecting Caprine encephalitis arthritis in these breeds by Nested PCR method.

Methods and Materials

Sampling and DNA Extraction

This study was carried out in a goat breeding flock in one of the west province of Iran (Figure 1), 249 blood samples were taken in tubes containing K2 from 86 heads of Saanen, 80 heads Alpine and 83 heads Indigenous goat breeds, and the age, gender, clinical symptoms for arthritis, mastitis, and encephalitis of each goat was recorded.

Fig 1. Map of Iran and the location of the epidemiologic unit sampled (26).

The blood samples were transferred to the laboratory and centrifuged at 5000 rpm for 5 minutes and after that, their Buffy coat was separated and transferred to sterile micro tubes, and finally the DNA of the samples was obtained using a DNA extraction kits (Iran, ROJE and BEHGENE) according to their instructions. The extracted DNA was evaluated with NanoDrop (Thermo SCIENTIFIC) and its purity was checked at 260/280 and 230/260 nm wavelengths and kept at -20 temperature.

In this study, two pairs of primers were used, whose target is the gag gene of CAEV virus (9) and NCBI Gene Bank Primer-Blast software was used to analyze the primers (Table 1). In this test, primers F1 and R1 were for the first stage of PCR and primers F2 and R2 were for nested PCR.

Table 1. Primers used for PCR and nPCR

Primer	Sequence 5′- 3′	Length of PCR product	Length of nPCR product
Gag F1	CAAGCAGCAGGAGGGAGAAGCTG	296 bp	
Gag R1	TCCTACCCCCATAATTTGATCCAC		
Gag F2	GTTCCAGCAACTGCAAACAGTAGCAATG		185 bp
Gag R2	ACCTTTCTGCTTCTTCATTTAATTTCCC		

Detection of GAG Gene by Polymerase Chain Reaction

To detect the gag gene, nested PCR method, which consists of two steps, was performed with two pairs of synthesized primers (Sinaclon, Iran). For this purpose, the extracted DNA was used as a template for proviral DNA detection. In the PCR stage, 3 µl of template DNA from each sample was added to Master Mix buffer (Amplicon, Denmark), which includes: Tris-HCL(pH8.5), (NH4)2SO4, 1.5 mM MgCl2, 0.25% Tween 20, 0.4mM dNTP, 1 µl of each primer and DNA polymerase Taq enzyme unit. The total reaction volume was adjusted to 25 µL with distilled water. For Nested PCR, the protocol of Fieni et al. was used, which was modified by Konishi et al.(25, 26). Then DNA was amplified by a thermal cycler (TC-TE, USA) with a program that includes: one cycle at 94°C for 5 minutes, 34 cycles that include denaturation for 30 seconds at 94°C, annealing at 55°C for 30 seconds, amplification at 72°C for 90 seconds and final amplification at 72°C for 5 minutes. Next, 3 µl of the amplified products were used for Nested PCR and continued with the primers for this stage and the same protocol as the first stage. The final PCR products were electrophoresed in 2% agarose gel. The final amplified product of nested PCR with a size of 184 bp was observed by GEL DOC imaging system (BIO-RAD).

Results

Nested PCR test results showed that out of the total number of 249 examined goats, 21 (8.3%) were positive for Saanen, Alpine and Indigenous breeds. (Table 2, Figure 2) and this ratio was 11 (13.8%), 7 (8.1%) and 3 (3.6%) for Alpine, Saanen and Indigenous breeds, respectively (Table 3).

Based on the age, the infection rate in goats less than 2 years, 2-3 years and more than 3 years was determined as 3 (15.8%), 7 (8%) and 11 (7.7%) respectively (Table 4).

Table 2. CAEV infection rate of goats based on Nested PCR method

Total	Nested PCR test result		
	Number of positive samples	The number of negative samples	Positive percentage
249	21	228	8.43

Table 3. The rate of CAEV infection in goats based on breed

breed	Total	Nested PCR test result	
		infected	percentage
Saanen	86	7	8.1
Alpine	80	11	13.8
Indigenous	83	3	3.6

Table 4. Correlation between infection rate and age in goats infected with CAEV

age/year	Number	infected	Percentage
≤2	19	3	15.8
2–3	87	7	8
≥3	143	11	7.7

Discussion

Our study was the first study in Iran that proved the prevalence of CAEV in imported dairy breeds and indigenous breeds using the Nested PCR method.

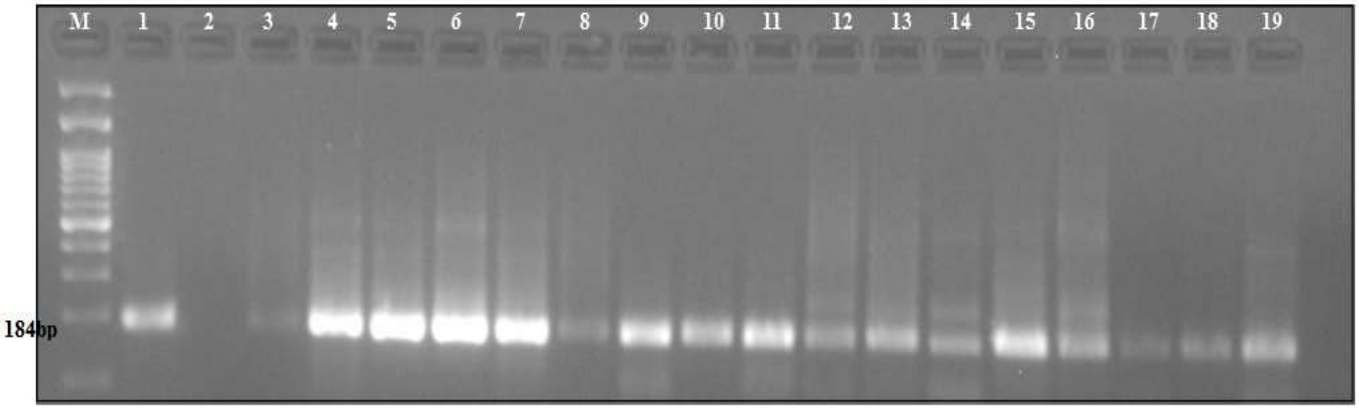

Fig 2. Image of Nested PCR test result step. Lane1: positive control, Lane2: negative control, lanes 3 to 20 positive CAEV samples, 184 bp fragments were amplified after the Nested PCR.

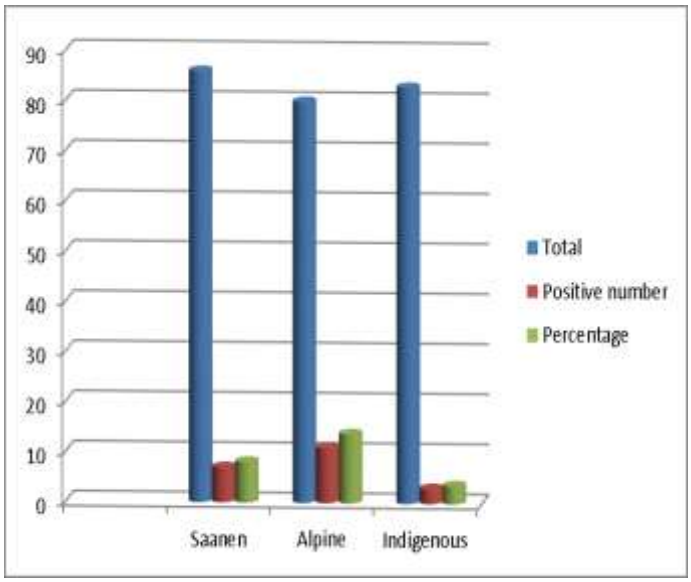

Fig 2. CAEV infection diagram of Saanen, Alpine and Indigenous

CAEV virus was first isolated in 1980 from a goat with arthritis (21). The prevalence of variation in the world has a high level of difference, ranging from less than 1% in Switzerland to 82% of tested herds in Australia (30, 31) While this disease was reported for the first time in Iran in 1390 and then 1392 (16, 27) based on the ELISA method, but due to the long interval between antibody production and detection in serological methods, the importance of PCR method with rapid infection detection has been revealed by Zanoni et al. (28, 29).

The overall prevalence of CAEV in this study was 8.43%, which was slightly different with Rahimifard 10.87% (27) , Moazed 14.67% (32) because both of them were worked on Elisa in indigenous goat and Koujori 15.7% (16) who was detected by PCR in indigenous goats were involved to arthritis and above all our study was in three breeds and used nested PCR. From the total of 249 samples related to Saanen, Alpine and Indigenous breeds that were analyzed by Nested PCR method, 8.1% (7), 13.8% (11) and 3.6% (3) were positive respectively, and the other hand, prevalence of CAEV in breeds Saanen, Alpine and Arabia breeds was less compared to the results of Algeria with 10%, 46.91% and 32.34% respectively and reason of this difference may be back to source of imported goats to our country (44). Although, Schultz et al have identified various factors in the eradication and resistance to CAE disease in two breeds of Saanan and Alpain (46).

Although our results were lower than many countries; Australia 82% (31), Taiwan 61.7% (8), the United States 45% (33), Switzerland 42% (34), Spain 23.22% (35), Lebanon 13.5% (36), Japan 10% (26), but it was more than Brazil 6.2% (37), Somalia 6% (38), Iraq 5.8% (12), Oman 1.5% (39), India 3.3% (40) and equally with Turkey 8.5% (41). For explain of this differences, methods to detect infectious rate (ELISA, PCR, Nested PCR, …), variation of breeds, age of animals were studies must be completely considered. CAEV remains latent in the body despite the immune system reaction and the production of an immune response, and seroconversion may take a long

time (42). As age increases, the chance of infection with goat arthritis encephalitis virus increases with horizontal transmission because the possibility and probability of contact is greater, and this justifies infection with CAEV at any age (43).

In our study, the mean age of infection was 3.3 years and the highest percentage of infection was seen at the age of less than 2 years15.8%, 2-3 (8%) years, more 3 years (7.7%). The results of this study and previous studies show CAEV infection both in Indigenous breeds that are kept in traditional methods and in dairy breeds that are raised industrially. Nested PCR method with high sensitivity and accuracy is efficient for detecting infected cases and in the absence of a suitable vaccine, it can help to identify and eliminate positive cases.

Conclusions

This study gives us an overview of the CAEV infection rate in dairy breeds imported to Iran as well as the Indigenous breed, and while announcing the risk of outbreak, it points out the need to intensify quarantine and hygiene conditions.

Acknowledgment

We are grateful for the guidance and efforts of Dr. Elahe Alivaisi at Ilam University, Kosar Khezri at Ilam University of Medical Sciences and Ali Allahmoradi at Agricultural and Natural Resources Research Center.

Disclosure

None

Conflict of Interest

No conflict of interest is declared.

Funding

None

Ethics Approval and Consent to Participate

None

Data Availability Statement

All data generated or analyzed during this study are included in this article.

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