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Initial multitarget approach shows importance for improved Caprine arthritis-encephalitis virus control program in Russia for hobbyist goat farms

Abstract

Aim: The aim of this study was to use a multi target approach to testing with both serological tests and an in-house real-time molecular test to investigate the prevalence of the caprine arthritis-encephalitis virus (CAEV) in goats from three hobbyist farms in the Republic of Tatarstan, Russia.

Materials and Methods: We have approached the detection of using a multi target approach testing with both ELISA and an in-house real-time PCR test to investigate the prevalence of CAEV in goats. Animals from three hobbyist farms were used in this study. The animals from two farms (n=13 for F1 and n=8 for F2) had clinical signs of arthritis and mastitis. In the third farm (n=15 for F3), all goats were homebred and had no contact with imported animals.

Results: CAEV antibodies (ELISA targets TM *env* and *gag* genes) were detected in serum samples from two farms (F1 and F2), indicating a seroprevalence 87.50-92.31%. Specific CAEV antibodies were also detected in milk samples. CAEV proviral DNA was detected in 53.85-62.50%. Results from all tests performed in the third farm (F3) were negative, indicting all tests were 100% specific.

Conclusion: Results of this work show that CAEV is circulating and present in small hobbyist goat farms in Russia. Serological and molecular tests could be of importance for CAEV control and eradication programs in Russia for hobbyist goat farms.

Keywords: caprine arthritis-encephalitis virus; goat; antigens; antibodies; proviral DNA.

Introduction:

Caprine arthritis-encephalitis virus (CAEV) belongs to the small ruminant lentiviruses (SRLVs), the genus Lentivirus, and the family Retroviridae, and can cause serious economic problems for goat farms. The infection may develop into multisystemic inflammatory diseases, which affect the central nervous system in kids and joints and mammary glands in adult goats [1,2]. However, the asymptomatic period may last several months or more. The virus (CAEV) was initially isolated in the United States from an infected adult goat more than 40 years ago [3]. Since that initial report the prevalence of CAEV has been reported in many countries [4-10]. Several reports describe the detection of specific CAEV antibodies in Russian goat populations, what indicates the circulation of CAEV in Russian goat farms [11-14]. In Belgium, small ruminant lentiviruses, including CAEV, were detected in small numbers of sheep and goats on hobbyist farms in the presence of an ongoing voluntary testing scheme [15], indicating that a low uptake on the voluntary scheme, can create difficulties and slows progress in the control program by harboring undetected seropositive animals. While, the un-proportionally high seroprevalence of CAEV in dwarf goats in reported in Switzerland indicates that these hobby breeds do not fall under official controls [16] and are going undetected, however, these hobby breeders are more likely to inadvertently escape some of the official controls. A widespread of CAEV infection in goat herds in southern Spain has been reported to be associated with such factors as, herd size, existence of kidding area, absence of cleaning and disinfection program, natural mating and multiparous births [5].

A major route for the spread of CAEV infection is, colostrum and milk from a seropositive goat, in these secretions free virus and infected macrophages or epithelial cells can be present [17,18]. Cross-species transmission of CAEV was also observed in wild small ruminants [19].

There is no “gold standard diagnostic test” available currently for CAEV, and use of a multi-faceted screening approach using both serological and molecular biology techniques for blood and milk samples is recommended to detect positive animals [20,21]. In chapter 3.7.2 of the OIE Terrestrial manual [22], the use of different diagnostic methods, including serology and PCR are recommended along with clinical evaluation and post mortem examinations for diagnosis of this persistent infection. Since CAEV is a life-long infection, animals are considered carriers and present as persistently seropositive animals.

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Antibody responses during CAEV infection do not play a protective role [23], but can be used for diagnostic purposes. In the humoral immune response of goats' immunoglobulins subtype IgG1 is the dominant type in infected goats with clinical arthritis and inflammatory joint lesions [24]. During the SRLV infection process antibodies against several antigens develop, including capsid protein p25CA, transmembrane protein gp46TM, nucleocapsid p14NC, matrix protein p16MA and surface protein gp135SU [25]. Due to this antigenic heterogeneity, using all or most of these antigens has the potential to increase the sensitivity of CAEV serodiagnosis [26-28].

CAEV, like all members of the retrovirus family, is an RNA-containing pathogen that upon infection of an organism, integrates a proviral insertion in the genome of an infected animal, and both provirus and virus detection can be achieved by PCR and RT-PCR, respectively [29,30]. CAEV detection methods based on defining the proviral insertion allows for the most expedient approach for its detection. CAEV was detected using nested PCR in the Philippines [5] and Argentina [9] previously. For the detection of proviral CAEV DNA, recombinase polymerase amplification (RPA) and a lateral flow dipstick (LFD) assay was recommended for use in another study [31]. Detecting CAEV proviral DNA in goat samples could be useful in eradication programs and epidemiological studies.

In Russia, the goat sector is small and consists mostly of hobbyist farmers keeping small numbers of animals on each farm. The current lack of CAEV prevalence data in this hobbyist sector makes it difficult to evaluate the risk of CAEV transmission, even for such relatively low farm and animal numbers. Here, we report the prevalence of CAEV in goats from three hobbyist farms as determined by ELISA and real-time PCR in the Republic of Tatarstan, Russia.

Materials and Methods:

Animals and clinical samples

Our research was conducted in three hobbyist goat farms in the Republic of Tatarstan, Russia, in 2015, during common veterinary examination of farms. Animals from two farms (n=13 for F1, n=8 for F2) containing a mixture of home bred and purchased animals some of which were already showing clinical signs of arthritis (Figure 1) and mastitis were used here for the assessment of infection prevalence. Goats from a third farm (n=15 for F3) were used to determine the tests apparent specificity, were all homebred and had no contact with imported animals, there were no clinical signs of CAEV observed. No CAEV testing history was available for animals used in this study.

Whole blood was collected into 4.5 mL Vacuette® K3E K3EDTA 13x75 lavender cap-black ring, premium tubes and also into Vacuette® Tube 4.5 ml Z Serum Clot Activator 13x75 red cap-black ring, premium tubes (Greiner Bio-One GmbH, Austria) from the jugular vein using Vacuette® Multiple Use Drawing Needles, 18G x 1 1/2" pink, sterile, latex-free, 1.25x38 mm (Greiner Bio-One GmbH, Austria). The serum was separated in the serum clot activator tubes by centrifugation at 500 g for 15 mins. Whole blood and serum samples stored at -20°C.

Milk samples were collected in glass tubes (Khimlaborpribor, Russia) and then stored at 2-8°C for 24 hrs. Samples were then centrifuged at 500 g for 15 mins, defatted, and then stored at -20°C. Bioethics Committee of Federal Center for Toxicological, Radiation and Biological Safety provided full approval for this research. A special ethical approval was not required, because animals were not involved in an experimental study. Only blood and milk samples, collected by veterinarians, were used. The samples taken did not exceed the volume that would have been taken for routine veterinary/animal husbandry purposes.

Figure 1. Clinical signs of arthritis in goats from farms F1 (A, B) and F2 (C).

Antibody detection

Goat serum and milk samples were tested using the commercial ELISA Maedi-Visna/CAEV Antibody Test Kit (IDEXX, France) with one modification for milk testing. This ELISA uses a mixture of a synthetic peptide of the immunogenic region of the transmembrane protein (TM *env* gene) and recombinant p28 protein, which is a part of the viral capsid (*gag* gene), immobilized as an antigen in the wells of ELISA plate. Briefly, serum samples were diluted 1:20 and individual milk samples were diluted 1:50 in dilution buffer and mixed before following the manufacturer's instructions for the ELISA test for serum samples. Results were analyzed according to the manufacturer's instructions and presented as S/P %.

Extraction of nucleic acids

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For the extraction of nucleic acids, 1ml of milk sample was placed into a centrifuge tube and centrifuged at 7,000 rpm (MiniSpin, Eppendorf) for 5 min, 800 µL of supernatant was removed, and the remaining 200 µL was used for DNA extraction. Whole blood samples were used without centrifugation.
The AmpliPraym DNA-sorb-B kit (NextBio) was used in according to the manufacturer's instructions for the isolation of DNA from milk and whole blood samples. Lysis solution (300 µL) and 100 µL of milk or whole blood sample were used per extraction.
The extracted DNA concentration and purity was measured using UV5Nano spectrophotometer (Mettler Toledo) according to the manufacturer's instructions. Samples of nucleic acids were stored at -80°C until use.

Design of oligonucleotide primers

For the in-house real-time PCR assay, the target site from CAEV proviral DNA, available in the GenBank (GenBank accession number: NC_001463), was selected using AlignX (ClustalW) and Vector NTI Version 9.1 (Invitrogen) programs. Several isolates/strains of the virus were analysed to identify a portion of the DNA that was homologous across all (Figure 2). The env gene was selected and using Standard Nucleotide BLAST (https://blast.ncbi.nlm.nih.gov) and a region between 7975-8098 bp (GenBank accession number: NC_001463) was identified as highly specific and primers and probes were designed within this region.

Figure 2. Homologous portion of the proviral DNA or viral RNA in CAEV isolates / strains with positions in CAEV genome shown.

The primer set (forward FCAEV; reverse RCAEV) and probe (PCAEV) used in this study (Table 1) were designed based on the CAEV proviral DNA specific region identified as above. Using Standard Nucleotide BLAST, it was confirmed that in this format no cross-reaction with DNA of other organisms can be observed and it is 100% specific to CAEV. The Oligo Analysis module of the Vector NTI was used to check the primers in vivo before syntheses (eg. melting temperature (Tm), primer-dimer formation and primer self-complementarity). The maximum annealing temperature of the primers was 60.3°C for forward (F) and 60.3°C for reverse (R) primers. The annealing temperature of the probe (P) was 65.5°C. The designed primers were found not to form dimers, secondary structures or palindromes and had a GC composition of 40-60%. The primers and probe (labeled with reporter and quencher dye (ROX, BHQ2) at its 5' and 3' ends respectively) were synthesized by Syntol (Moscow, Russia) (Table 1).

Real-time PCR

Real-time PCR for CAEV proviral DNA was performed using a universal master mix RT-PCR kit (Syntol, Moscow, Russia), comprising: 25 mM MgCl2, 2.5 mM dNTP, PCR buffer ×10, Taq polymerase and deionized water. The final volume of 20 µL PCR mixture contained: 1.5 µL of 25 mM MgCl2 solution; 0.5 µL of 10 pM probe solution; 0.5 µL of 10 pM of each primer solution; 1.5 µL of 2.5 mM dNTP solution; 1.5 µL of 10x buffer for PCR; 0.5 µL of Taq polymerase; 10 µL of DNA extract and 3.5 µL of deionized water. PCR was carried out in real-time on amplification platform C1000 with an optical reaction module CFX96 (BioRad). The PCR cycling conditions were as follows: (I) denaturation at 95°C for 3 min followed by (II) 5 cycles of 10 sec each at 95°C and 30 sec at 60.0°C, and then (III) 39 cycles: 10 sec at 95°C, 30 sec at 60.0°C (acquisition of fluorescent signal).

Positive control

A synthetic insert as 150bp of synthetic DNA (5′gcaagtctgggagtcgcaaacgcgattcagcagtcctatactagggcggctgtccagacccttgctaatgcaactgctgcacagcaggatgtgttagaagc aacctatgccatggtacagcatgtggctaaaggcgtcaggatcttggaa3′) was designed to include recognition sites for CAEV gene (GenBank accession number: NC_001463) and was inserted into synthetic oligonucleotide sequences. The final nucleotide sequence was synthesized and then subcloned within plasmid pAL2-T (ZAO Evrogen, Russia), which was used as a positive control in real-time PCR for CAEV proviral DNA.

Statistical analysis

The prevalence was calculated using Wilson 95% confidence interval (CI) without a correction for continuity available on line (The Confidence Interval of a Proportion/VassarStats: http://vassarstats.net/prop1.html). The

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agreement between tests were calculated using Kappa available on line (Kappa as a Measure of Concordance in Categorical Sorting/VassarStats: http://vassarstats.net/kappa.html).

Results:

Serorevalence of CAEV

For comparison of CAEV seroprevalence in the two herds where animals were showing clinical symptoms of CAEV infection (Figure 1), results from F1 and F2 were analised. In the cases of F1 and F2, 12/13 and 7/8 goats were positive in IDEXX assay, with seroprevalence 92.31% (95% CI 66.69 to 98.63%) and 87.50% (95% CI 52.91 to 97.76%), respectively. For comparison of apparent specificity, 15 goats from F3 were tested for presence of specific CAEV antibodies in serum samples using the Maedi-Visna/CAEV Antibody Test Kit (IDEXX). None of these 15 goats were antibody positive by the ELISA (Table 2).

Prevalence of CAEV antibody in milk

Positive ELISA results were obtained for 9/10 milk samples from goats of F1, (90.00%, 95% CI 59.59 to 98.21%). For 2/10 animals there were no clinical signs, but were determined to be serum positive. Antibodies were detected in one sample obtained from a goat with clinical symptoms (33.33%; 95% CI 6.15 to 79.23%). Only 5/15 milk samples from F3 were tested with the ELISA, and all five were negative.

Comparison of serum and milk

While the observed agreement between serum and milk test results for IDEXX for the three farms (total n= 18) was 0.7692 indicating good agreement. However, serum ELISA testing detected more positive goats, than milk samples tested here.

Synthetic viral DNA detection limit

To determine the detection limit of the PCR, synthetic viral DNA was titrated (13 tenfold dilutions) and tested. The initial concentration of the DNA was 426.6 ng/µL (plasmid DNA 3150bp; 1×10^{14} copies/mL). Real-time PCR was carried out on the dilutions, starting at the 1×10^{11} copies/mL. Each dilution was amplified and results confirmed for 8 repeats per dilution (Figure 3).

Figure 3. Real-time PCR result of plasmid DNA containing marker DNA CAEV. Amplification curves of CAEV, respectively using serial 10-fold dilutions of the mixed recombinant plasmids from, 1×10^{11}, 1×10^{10}, 1×10^{9}, 1×10^{8}, 1×10^{7}, 1×10^{6}, 1×10^{5}, 1×10^{4}, 1×10^{3} and 100 copies/mL.

The last dilution successfully amplified was 1×103 copies/mL (1×10 copies/10µL), at this dilution a positive reaction was observed in 6 out of 8 of the repeats while all of the dilution 1×104 copies/mL (1×102 copies/10µL) were amplified in all cases, the dilution 100 copies/mL (1×1 copies/10µL) did not amplify. To evaluate the inter-assay variability, tenfold dilutions of 1×10^{12}, 1×10^{11}, 1×10^{10}, 1×10^{9}, 1×10^{8}, 1×10^{7}, 1×10^{6}, 1×10^{5}, 1×10^{4}, 1×10^{3} and 100 copies/mL of the plasmid DNA were tested (8 replicates on the same amplification run). The CV values of intra-assay were 0.1 – 14.88 (Table 3).

Real-time PCR for CAEV proviral DNA

The total DNA concentration range for both milk and whole blood-based extractions were in the range of 200 to 1.200 µg/mL, and all DNA extracted in this study had 260/280 ratio of >1.8 (data not shown).
In order to determine the detection limit for the PCR assay, serial dilutions were prepared based on total DNA extracted from a whole blood sample. Serial dilutions of total goat DNA from 900 to 0.05 µg/mL (Figure 4) were used. The Cq at the lowest DNA dilution (0.05ug/mL total DNA) was determined as 37.57.

Figure 4. Real-time PCR result of goat F1-11 milk sample: total DNA titration from 900 to 0.05 µg/mL (900µg/mL, 300µg/mL, 100µg/mL, 33µg/mL, 11µg/mL, 3.7µg/mL, 1.2µg/mL, 0.41µg/mL, 0.05µg/mL) and no amplification negative control.

Whole blood samples from all 15 goats from F3 were tested and 5 were tested by the milk-based PCR, in both cases all samples were PCR negative (Table 2).

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A total of 13 blood samples from F1 were tested by real-time PCR and CAEV proviral DNA was detected in 7/13 animals (Table 2) with prevalence 53.85% (95% CI 29.15 to 76.80%). 5 of these 7 goats had clinical signs of CAEV infection (arthritis), there were no clinical signs in the other 2 animals. Real-time PCR was carried out for 10 milk samples from these 13 animals. CAEV proviral DNA was detected in 4/10 milk samples. These 4 animals were also positive when blood was PCR tested.

In F2 blood samples from 8 goats were tested and PCR was positive in 5/8 samples, indicating the prevalence of CAEV proviral DNA to be at 62.50% (95% CI 30.57 to 86.32%). Of the 5 animals tested as positive, 3/5 had clinical CAEV symptoms.

Milk samples from F2 were also tested for three animals, using real-time PCR. CAEV proviral DNA was detected in 1/3 of these milk samples. This one positive animal was also positive when blood was tested by PCR.

CAEV proviral DNA was not detected in any blood or milk samples from F3, where clinical signs of CAEV were absent in all animals.

These results indicate that used in this study real-time PCR is highly sensitive and it can detect CAEV proviral DNA at very low concentration (Figure 3).

Comparison of ELISA and PCR

An overall good level of agreement of 0.6182, was determined for samples (milk/serum/blood) from all three farms (n=36) between all tests done on the IDEXX ELISA and the PCR.

Discussion:

There are no official reports of CAEV infection in goats on hobbyist farms and no eradication/monitoring programs exist currently, in Republic of Tatarstan, Russia. There is a goat/sheep population of 65.8 thousand heads as of 2016 [32], of which it is estimated that 90.5% are in small and hobbyist farms. Currently control measures consist of veterinary certification of imported goats as originating from a CAEV-free region only [33], which aims to prevent the introduction of the infection to farms in Russia. However, it is common practice for large dairy goat farmers to strictly control and test for a range of different infections to help prevent entry of any infected animals into their herds. Goats from three small hobbyist farms in the Republic of Tatarstan, Russia, were investigated here for the presence of CAEV antibodies and proviral DNA. CAEV antibodies were detected in serum samples from two farms, where animals were also showing clinical signs of the disease, with seroprevalence at levels of 92.31% (95% CI 66.69 to 98.63%) in Farm 1 and 87.50% (95% CI 52.91 to 97.76%) in Farm 2 determined. Specific CAEV antibodies were also detected in milk samples from the two farms (F1 and F2) indicating a prevalence of 90.00% (95% CI 59.59 to 98.21%) and 33.33% (95% CI 6.15 to 79.23%), respectively. CAEV proviral DNA was also detected with levels of 53.85% (95% CI 29.15 to 76.80%) and 62.50% (95% CI 30.57 to 86.32%), for the two farms respectively. The observed agreement between serum and milk ELISA results was 0.7692, and between ELISA and PCR was 0.6182 in the two farms. This indicates the presence of previously unreported CAEV seropositive goats in these farms in Republic of Tatarstan, Russia.

It has been demonstrated in an Italian study that ELISA can be used as a diagnostic test for control measures, for aiding the reduction of seroprevalence as well as clinical manifestations of CAEV infection [34]. The reactivity of CAEV antigens in serological tests may vary which depends on the geographical and breeding origin of the goats [35]. The presence of antibodies and proviral DNA in goat samples may vary within the time, and the combination of different tests for CAEV diagnostics may improve the efficacy of control and eradication programs [20,21,36]. Therefore, for epidemiological purposes, serological assays with various CAEV antigens as well as PCR methods are needed for detection of the disease. The study in Thailand showed that combination of ELISA and PCR provided advantages to detect CAEV-infected goats [37]. The IDEXX ELISA kit, which was used in our study, uses a mixture of the transmembrane protein (TM ENV gene) and recombinant p28 protein (GaG gene) for detection of the infection as a multi-antigen approach. It was reported that monoclonal antibodies against p28 are also reactive against p55 (gag) protein and the intermediate cleavage products, p44, p36 and p22 [38]. The antibody response was significantly higher among arthritic than asymptomatic goats [28]. In our study, all animals with clinical signs of CAEV were seropositive.

There is potential for using PCR for CAEV, as an alternative to serology or as a supplemental test, CAEV RNA PCR and previously a PCR for detection of CAEV proviral DNA was shown to be highly efficient [39]. It was also reported that presence of CAEV proviral-DNA and CAEV in the seminal plasma was significantly higher in bucks with PCR-positive blood [40]. Proviral DNA was detectable 15 days post experimental CAEV

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infection, whereas specific antibodies were detected after 40-60 days using a real time PCR also targeting a specific region of the CAEV env gene [41]. The CAEV genome is characterized by a pronounced polymorphism of the nucleotide sequence. In the conservative sequence we have chosen [42] to indicate the CAEV provirus the polymorphism is also observed (Figure 2), and the oligonucleotides for CAEV indication are complementary to non-polymorphic regions of this sequence.

Results from this study show that the use of both ELISA and PCR has the advantage of potentially improving the sensitivity. However, the lack of agreement between ELISA and PCR results reported by other authors [20,21] has also been observed in this study. The results of this study revealed a pattern of a positive reaction in ELISA and PCR (both with blood samples and milk) in goats with clinical manifestations of the disease (samples: F1-6, F1-7, F1-11 and F2-1). This circumstance is associated with the peculiarities of CAEV infection process, the provirus can be detected at early stages and during virus release into the environment, antibodies are synthesized at a later stage after infection and can circulate in the organism of infected animals for a long time. Recently it was reported that goats on multispecies farms (where goats and sheep coexist) in Italy had a higher CAEV seroprevalence, where sheep can serve as a reservoir of small ruminant lentivirus infection [43]. Also indications for cross-species transmission of small ruminant lentivirus strains between sheep and goats were found in Belgium [44]. Hobbyist farms in most cases are multispecies farms, and small ruminant lentivirus control programs should be concentrated on both sheep and goats. The lack of regular screening for small ruminant lentiviruses is increasing the spread of the disease [45-47].

The results of our studies on the example of three small farms in the Republic of Tatarstan indicate the possibility of the spread of CAEV among goats on hobbyist farms, the owners of which lack the professionalism to control this viral disease. Moreover, the absence of reported CAEV cases in large goat farms indicates compliance with veterinary and animal husbandry regulations. Initial work presented here on the in-house PCR show that the test is specific in the samples tested, however further work is needed to develop the assay further for both milk and blood-based assay. The work on the analysis of the prevalence of CAEV in the Republic of Tatarstan and other regions of the Russian Federation will continue.

Conclusion:

The results of this study indicate that CAEV is circulating and present in small hobbyist goat farms in the Republic of Tatarstan, Russia and is currently going undetected in the absence of a control program or monitoring. Due to the complex nature of the CAEV infection and viral life cycle and based on the results in this comparative study it can be concluded that serological tests, targeting different proteins, as well as molecular based tests could be of importance for use in any future CAEV control and eradication programs in Russia for hobbyist goat farms.

References:

1. Larruskain A, Jugo BM. 2013. Retroviral infections in sheep and goats: small ruminant lentiviruses and host interaction. Viruses 5(8): 2043-2061 DOI: 10.3390/v5082043.
2. Ravazzolo AP, Nenci C, Vogt HR, Waldvogel A, Obexer-Ruff G, Peterhans E, Bertoni G. 2006. Viral load, organ distribution, histopathological lesions, and cytokine mRNA expression in goats infected with a molecular clone of the caprine arthritis encephalitis virus. Virology 350(1): 116-127.
3. Crawford TB, Adams DS, Cheevers WP, Cork LC. 1980. Chronic arthritis in goats caused by a retrovirus. Science 207(4434): 997-999.
4. Tabet E, Hosri C, Abi-Rizk A. 2015. Caprine arthritis encephalitis virus: prevalence and risk factors in Lebanon. Rev Sci Tech 34(3): 915-921,907-914.
5. Padiernos RB, Balbin MM, Parayao AM, Mingala CN. 2015. Molecular characterization of the gag gene of caprine arthritis encephalitis virus from goats in the Philippines. Arch Virol 160(4): 969-978 DOI: 10.1007/s00705-015-2359-5.
6. Linderot de Cardona K, De Gracia Scanapieco A, Braun PG. 2016. First results on small ruminant brucellosis and tuberculosis and caprine arthritis-encephalitis in El Salvador. Trop Anim Health Prod 48(5): 1083-1087 DOI: 10.1007/s11250-016-1044-3.
7. Konishi M, Hayama Y, Shirafuji H, Kameyama K, Murakami K, Tsutsui T, Akashi H. 2016. Serological survey of caprine arthritis-encephalitis virus infection in Japan. J Vet Med Sci 78(3): 447-450 DOI: 10.1292/jvms.15-0357.

8. Barrero Domínguez B, Luque I, Maldonado A, Huerta B, Sánchez M, Gomez Laguna J, Astorga R. 2017. Seroprevalence and risk factors of exposure to caprine arthritis-encephalitis virus in southern Spain. Vet Rec 180(9): 226 DOI: 10.1136/vr.104014.
9. Panei CJ, Gos ML, Valera AR, Galosi CM, Echeverria MG. 2017. First isolation and nucleotide comparison of the gag gene of the caprine arthritis encephalitis virus circulating in naturally infected goats from Argentina. Open Vet J 7(1): 32-35 DOI: 10.4314/ovj.v7i1.5.
10. Yang WC, Chen HY, Wang CY, Pan HY, Wu CW, Hsu YH, Su JC, Chan KW. 2017. High prevalence of caprine arthritis encephalitis virus (CAEV) in Taiwan revealed by large-scale serological survey. J Vet Med Sci 79(2): 273-276 DOI: 10.1292/jvms.16-0387.
11. Sidelnickov GD, Colbasov DV, Chichickin AJ, Volkova IJ, Rzhavin AV. 2007. Experience in sanitation of a caprine arthritis-encephalitis-affected farm. Veterinariya 12: 25-26. (in Russian).
12. Kudryashov AA, Balabanova VI, Babina SY. 2014. Pathomorphological findings in lungs and brain at caprine arthritis-encephalitis. Actual Questions of Veterinary Biology 3: 54-58. (in Russian)
13. Baryshnikova EI, Kolbasova OL, Malogolovkin AS. 2015. Investigation of molecular genetic characteristics of small ruminant lentivirus. Voprosy Normativno-Pravovogo Regulirovaniya v Veterinarii 2: 27-28. (in Russian)
14. Balabanova VI, Kudriashov AA, Shumakov OP. 2016. Pathologic diagnosis of contagious diseases of goats and sheep on farms. Actual Questions of Veterinary Biology 4(32): 43-48. (in Russian)
15. Michiels R, Van Mael E, Quinet C, Welby S, Cay AB, De Regge N. 2018. Seroprevalence and risk factors related to small ruminant lentivirus infections in Belgian sheep and goats. Prev Vet Med 151: 13-20 DOI: 10.1016/j.prevetmed.2017.12.014.
16. Thomann B, Falzon LC, Bertoni G, Vogt HR, Schüpbach-Regula G, Magouras I. 2017. A census to determine the prevalence and risk factors for caprine arthritis-encephalitis virus and visna/maedi virus in the Swiss goat population. Prev Vet Med 137(Pt A): 52-58 DOI: 10.1016/j.prevetmed.2016.12.012.
17. Blacklaws BA, Berriatua E, Torsteinsdottir S, Watt NJ, de Andres D, Klein D, Harkiss GD. 2004. Transmission of small ruminant lentiviruses. Vet Microbiol 101(3): 199-208.
18. Reina R, Berriatua E, Luján L, Juste R, Sánchez A, de Andrés D, Amorena B. 2009. Prevention strategies against small ruminant lentiviruses: an update. Vet J 182(1): 31-37 DOI: 10.1016/j.tvjl.2008.05.008.
19. Minardi da Cruz JC, Singh DK, Lamara A, Chebloune Y. 2013. Small ruminant lentiviruses (SRLVs) break the species barrier to acquire new host range. Viruses 5(7): 1867-1884 DOI: 10.3390/v5071867.
20. Barquero N, Arjona A, Domenech A, Toural C, de las Heras A, Fernández-Garayzabal JF, Ruiz-Santa Quiteria JA, Gomez-Lucia E. 2011. Diagnostic performance of PCR and ELISA on blood and milk samples and serological survey for small ruminant lentiviruses in central Spain. Vet Rec 168(1): 20 DOI: 10.1136/vr.c4951.
21. Barquero N, Gomez-Lucia E, Arjona A, Toural C, Heras A, Fernández-Garayzabal JF, Domenech A. 2013. Evolution of specific antibodies and proviral DNA in milk of small ruminants infected by small ruminant lentivirus. Viruses 5(10): 2614-2623 DOI: 10.3390/v5102614.
22. Caprine arthritis/encephalitis and Maedi-visna. Manual of Diagnostic Tests and Vaccines for Terrestrial Animals 2019. Chapter 3.7.2.
23. Blacklaws BA. 2012. Small ruminant lentiviruses: immunopathogenesis of visna-maedi and caprine arthritis and encephalitis virus. Comp Immunol Microbiol Infect Dis 35(3): 259-269 DOI: 10.1016/j.cimid.2011.12.003.
24. Trujillo JD, Hötzel KJ, Snekvik KR, Cheevers WP. 2004. Antibody response to the surface envelope of caprine arthritis-encephalitis lentivirus: disease status is predicted by SU antibody isotype. Virology 325(1): 129-136.
25. Torsteinsdóttir S, Carlsdóttir HM, Svansson V, Matthíasdóttir S, Martin AH, Pétursson G. 2007. Vaccination of sheep with Maedi-visna virus gag gene and protein, beneficial or harmful? Vaccine 25(37-38): 6713-6720.
26. Grego E, Bertolotti L, Carrozza ML, Profiti M, Mazzei M, Tolari F, Rosati S. 2005. Genetic and antigenic characterization of the matrix protein of two genetically distinct ovine lentiviruses. Vet Microbiol 106(3-4): 179-85 DOI: 10.1016/j.vetmic.2004.12.007.
27. Tolari F, Al-Ramadneh W, Mazzei M, Carrozza ML, Forzan M, Bandecchi P, Grego E, Rosati S. 2013. Small ruminant lentiviruses in Jordan: evaluation of sheep and goat serological response using recombinant and peptide antigens. Trop Anim Health Prod 45(6): 1335-40 DOI: 10.1007/s11250-013-0366-7.
28. Czopowicz M, Szaluś-Jordanow O, Moroz A, Mickiewicz M, Witkowski L, Markowska-Daniel I, Bagnicka E, Kaba J. 2018. Use of two commercial caprine arthritis-encephalitis immunoenzymatic assays for screening of arthritic goats. J Vet Diagn Invest 30(1): 36-41 DOI: 10.1177/1040638717729397.

29. Turchetti AP, Paniago JJ, da Costa LF, da Cruz JC, Braz GF, Gouveia AM, Paixão TA, Santos RL, Heinemann MB. 2013. Distribution of caprine arthritis encephalitis virus provirus, RNA, and antigen in the reproductive tract of one naturally and seven experimentally infected bucks. Theriogenology 80(8): 933-9 DOI: 10.1016/j.theriogenology.2013.07.021.

30. Marinho RC, Martins GR, Souza KC, Sousa ALM, Silva STC, Nobre JA, Teixeira MFS. 2018. Duplex nested-PCR for detection of small ruminant lentiviruses. Braz J Microbiol 49 Suppl 1: 83-92 DOI: 10.1016/j.bjm.2018.04.013.

31. Tu PA, Shiu JS, Lee SH, Pang VF, Wang DC, Wang PH. 2017. Development of a recombinase polymerase amplification lateral flow dipstick (RPA-LFD) for the field diagnosis of caprine arthritis-encephalitis virus (CAEV) infection. J Virol Methods 243: 98-104 DOI: 10.1016/j.jviromet.2017.01.023.

32. Federal State Statistics Service. Results of the 2016 All-Russian Agricultural Census: statistical compilation. Tatarstanstat, Kazan, 2018. Vol. 5. The population of farm animals. 560 p. https://tatstat.gks.ru/folder/33497 (in Russian)

33. Order of the Ministry of Agriculture of Russia dated 09.03.2011 N 62 (as amended on 15.02.2017) On approval of the List of contagious and other animal diseases. https://legalacts.ru/doc/prikaz-minselkhoza-rf-ot-09032011-n-62/ (in Russian).

34. Tavella A, Bettini A, Ceol M, Zambotto P, Stifter E, Kusstatscher N, Lombardi R, Nardeli S, Beato MS, Capello K, Bertoni G. 2018. Achievements of an eradication programme against caprine arthritis encephalitis virus in South Tyrol, Italy. Vet Rec 182(2): 51 DOI: 10.1136/vr.104503.

35. Bertoni G, Hertig C, Zahno ML, Vogt HR, Dufour S, Cordano P, Peterhans E, Cheevers WP, Sonigo P, Pancino G. 2000. B-cell epitopes of the envelope glycoprotein of caprine arthritis-encephalitis virus and antibody response in infected goats. J Gen Virol 81(Pt 12): 2929-2940 DOI: 10.1099/0022-1317-81-12-2929.

36. Gomez-Lucia E, Barquero N, Domenech A. 2018. Maedi-Visna virus: current perspectives. Vet Med (Auckl) 9: 11-21 DOI: 10.2147/VMRR.S136705.

37. Panneum S, Rukkwamsuk T. 2017. Diagnosis of Caprine arthritis encephalitis virus infection in dairy goats by ELISA, PCR and viral culture. Pol J Vet Sci 20(2): 347-353 DOI: 10.1515/pjvs-2017-0042.

38. Brandão CF, Campos GS, Silva AC, Torres JA, Tigre DM, Sardi SI. 2013. Monoclonal antibodies against Caprine arthritis-encephalitis virus epitopes in the p28 and p55(gag) viral proteins. J Virol Methods 187(2): 352-356 DOI: 10.1016/j.jviromet.2012.10.020.

39. Huang J, Sun Y, Liu Y, Xiao H, Zhuang S. 2012. Development of a loop-mediated isothermal amplification method for rapid detection of caprine arthritis-encephalitis virus proviral DNA. Arch Virol 157(8): 1463-1469 DOI: 10.1007/s00705-012-1322-y.

40. Ali Al Ahmad MZ, Fieni F, Pellerin JL, Guiguen F, Cherel Y, Chatagnon G, Bouzar AB, Chebloune Y. 2008. Detection of viral genomes of caprine arthritis-encephalitis virus (CAEV) in semen and in genital tract tissues of male goat. Theriogenology 69(4): 473-480 DOI: 10.1016/j.theriogenology.2007.10.017.

41. Brajon G, Mandas D, Liciardi M, Taccori F, Meloni M, Corrias F, Montaldo C, Coghe F, Casciari C, Giammarioli M, Orrù G. 2012. Development and field testing of a real-time PCR assay for Caprine arthritis-encephalitis-virus (CAEV). Open Virol J 6: 82-90 DOI: 10.2174/1874357901206010082.

42. Saltarelli M, Querat G, Konings DAM, Vigne R, Clements JE. 1990. Nucleotide sequence and transcriptional analysis of molecular clones of CAEV which generate infectious virus. Virology 179(1): 347-364. DOI: 10.1016/0042-6822(90)90303-9.

43. Nardelli S, Bettini A, Capello K, Bertoni G, Tavella A. 2020. Eradication of caprine arthritis encephalitis virus in the goat population of South Tyrol, Italy: analysis of the tailing phenomenon during the 2016-2017 campaign. J Vet Diagn Invest 32(4): 589-593. DOI: 10.1177/1040638720934055.

44. Michiels R, Adjadj NR, De Regge N. 2020. Phylogenetic analysis of Belgian small ruminant lentiviruses supports cross species virus transmission and identifies new subtype B5 strains. Pathogens 9(3): 183. DOI: 10.3390/pathogens9030183.

45. Potarniche AV, Cerbu CG, Czopowicz M, Szalus-Jordanow O, Kaba J, Spinu M. 2020. The epidemiological background of small ruminant lentivirus infection in goats from Romania. Veterinary World 13(7): 1344-1350. DOI: 10.14202/vetworld.2020.1344-1350.

46. Idres T, Lamara A, Temim S, Boudjellaba S, Gagnon J, Chebloune Y. 2019. Serological Diagnosis of Lentivirus Infection in Goats Raised in Algeria. J Vet Res 63(1): 27-33. DOI: 10.2478/jvetres-2019-0001.

47. Jesse FFA, Bitrus AA, Abba Y, Raju VN, Hambali IU, Peter ID, Haron AW, Lila MAM, Norsidin JM. 2018. Seroprevalence of small ruminant caprine arthritis encephalitis lentivirus among goats from selected small ruminant farms in Selangor, Malaysia. Veterinary World 11(2): 172-176. DOI: 10.14202/vetworld.2018.172-176.

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Tables:

Table 1. Primers and probe designed in this study.

Primers and probe	Sequence (5'-3')	Position in genome[1]	Amplicon size (bp)
FCAEV	TCGCAAACGCGATTCAGCAGT	7975-7995	124
PCAEV	ROX-CTGTCCAGACCCTTGCTAATGCAACTGC-BHQ2	8011-8038	
RCAEV	ACGCCTTTAGCCACATGCTGTACC	8075-8098	

[1] Numbering according to the Caprine arthritis-encephalitis virus, complete genome (GenBank accession number: NC_001463)

Table 2. Summary of all test results using the IDEXX Maedi-Visna/CAEV Antibody Test Kit and in-house real-time PCR assay.

Farm	Animal ID	CAEV Symptoms	IDEXX ELISA, Serum	PCR, Blood	IDEXX ELISA, Milk	PCR, Milk
Farm 1 (F1)	F1-1	Present	POS	POS	NEG	POS
	F1-2	Present	POS	NEG	POS	NEG
	F1-3	Present	POS	NEG	POS	NEG
	F1-4	Present	POS	POS	POS	NEG
	F1-5	Present	POS	NEG	POS	NEG
	F1-6	Absent	POS	POS	POS	POS
	F1-7	Absent	POS	POS	POS	POS
	F1-8	Present	POS	NEG	N/A	N/A
	F1-9	Present	POS	NEG	POS	NEG
	F1-10	Present	POS	POS	N/A	N/A
	F1-11	Present	POS	POS	POS	POS
	F1-12	Present	POS	POS	POS	NEG
	F1-13	Absent	NEG	NEG	N/A	N/A
Far 2 (F2)	F2-1	Present	POS	POS	POS	POS
	F2-2	Present	POS	POS	N/A	N/A
	F2-3	Absent	POS	POS	N/A	N/A
	F2-4	Present	POS	POS	N/A	N/A
	F2-5	Present	POS	NEG	N/A	N/A
	F2-6	Absent	POS	POS	N/A	N/A
	F2-7	Absent	NEG	NEG	NEG	NEG
	F2-8	Absent	POS	NEG	NEG	NEG
Farm 3 (F3)	F3-1	Absent	NEG	NEG	N/A	N/A
	F3-2	Absent	NEG	NEG	N/A	N/A
	F3-3	Absent	NEG	NEG	NEG	NEG
	F3-4	Absent	NEG	NEG	N/A	N/A
	F3-5	Absent	NEG	NEG	NEG	NEG
	F3-6	Absent	NEG	NEG	NEG	NEG

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F3-7	Absent	NEG	NEG	NEG	NEG
F3-8	Absent	NEG	NEG	N/A	N/A
F3-9	Absent	NEG	NEG	N/A	N/A
F3-10	Absent	NEG	NEG	N/A	N/A
F3-11	Absent	NEG	NEG	NEG	NEG
F3-12	Absent	NEG	NEG	N/A	N/A
F3-13	Absent	NEG	NEG	N/A	N/A
F3-14	Absent	NEG	NEG	N/A	N/A
F3-15	Absent	NEG	NEG	N/A	N/A

404 POS – positive result, NEG – negative result, N/A – not applicable

405 **Table 3.** Intra-assay for PCR detection of synthesized viral DNA.

Target	Conc. (copies/reaction)	Number of determinations	Mean Ct	CV
CAEV	1×10^9	8	6,38	0,827677
	1×10^8	8	10,98	0,535454
	1×10^7	8	14,86	0,157619
	1×10^6	8	18,21	0,103976
	1×10^5	8	21,57	0,182821
	1×10^4	8	24,84	0,1283
	1×10^3	8	28,49	0,414638
	1×10^2	8	31,19	0,515722
	1×10	8	33,75	14,61552
	1×1	8	N/A	N/A

406 Mean Ct – average value of the beginning of registration of amplification reaction,
407 CV – coefficient of variation, standard deviation from Mean Ct