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CLINICAL, PATHOLOGICAL AND MOLECULAR FINDINGS IN CAPRINE ARTHRITIS – ENCEPHALITIS VIRUS INFECTION IN DAMASCUS GOATS

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Caprine Arthritis-Encephalitis Virus Infection was presented with *clinical*, *pathological, and molecular findings* in dairy goats from a breeding unit in Turkey. According to history, 50 of 700 goats and kids showed the clinical signs including anorexia, mastitis, swelling of the joints, lameness, dyspnea and head pressing in the past 2 months. Fifteen and 6 animals were examined clinically and pathologically, respectively. Clinical examination revealed depression, weakness, pneumonia, ataxia and paresis in 15 goats. Histological lesions were composed by a varying degree of interstitial pneumonia, nonsuppurative leukoencephalomyelitis, interstitial nephritis and interstitial myocarditis. Immunohistochemical examinations showed the presence of viral antigen in tubular epithelium, in glial cells and macrophages.

In conclusion, this is the first report documenting pathological and molecular changes of Caprine Arthritis-Encephalitis Virus Infection in Turkey in goats.

Key words: Caprine Arthritis Encephalitis Virus, ELISA, goat, histopathology, PCR.

INTRODUCTION

Lentiviruses are grouped in genus within the family of *Retroviridae* and they are enveloped viruses having single-stranded RNA. 'Lenti prefix' means 'slow' (Latin), and these viruses cause life-long and deadly infections with prolonged incubation period in felids, primates and ungulates. Lentiviral infections cause either immunodeficiency or inflammatory changes. Unlike to other viruses, they can infect both dividing and non-dividing cells [1,2].

Caprine Arthritis Encephalitis Virus (CAEV) is a non-oncogenic retrovirus belonging to the subfamily of small ruminant lentiviruses. The viral genome has tropism for

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monocytes, macrophages, and dendritic cells. The virus can infect mainly mature macrophages producing cytokines that recruit and activate other leukocytes, and also enhance lentiviral replication [3]. Although the immune system supresses the viral replication to some degree during the incubation period, the virus replicates gradually in lymphoid cells. When the viral load reaches high enough, tissues damage and clinical signs might occur [1].

Clinical signs and pathological changes are the result of the inflammatory process instead of direct viral damage to the organ. The lesions of CAEV are the reflection of slow persistent inflammatory diseases and 4 pathological syndromes occur namely; encephalomyelitis, interstitial pneumonia, arthritis and mastitis [4].

After the first report of CAEV in the USA about five decades ago [5], the subsequent reports showed the prevalence of infection worldwide with the exception of New Zealand, Australia and Iceland [6]. Previous serological surveys by using ELISA in Turkey showed the prevelance of the infection ranging from 1.90% to 12.30% in different geographic regions, ages and breeds [7-10]. Although serological surveys are practical for screening herds, to recognize the specific clinical and pathological changes is also important in veterinary practice.

The purpose of this study was to present clinical and pathological changes of CAEV infection confirmed molecularly in a dairy goat farm.

MATERIAL AND METHODS

Animals

Fifteen Damascus goats and kids used in this study were obtained from a dairy farm and the animals were presented to Firat University Veterinary Medical Teaching Hospital for diagnosis and treatment. For pathological examination, 6 dead goats were delivered to the necropsy unit.

ELISA Analysis

For the ELISA test, blood samples were collected from the jugular veins of 15 goats and kids into non-anticoagulant tubes. The samples were centrifuged for 10 minutes at 3000 rpm and their sera were separeted to eppendorf tubes, which were stored at -20 °C until analysis. In addition, 10 blood samples (buffy coat) from animals showing clinical signs in the herd and for herd screening. ELISA test was performed from the blood for herd screening. Pourquier ELISA Maedi-Visna / CAEV Screening kit (Institute Pourquier, France) was used for this purpose [11]. This test was carried out according to the instructions of the manufacturer.

Necropsy

In addition to clinical examination of 15 goats, 6 dead goats were necropied. They were all female and they were approximately 3 to 4 years old. After necropsy, tissue samples from liver, lymph nodes, brain, lungs, kidneys and spleen were taken for histopathological and PCR examination.

Histopathological and Immmunohistochemical examinations

Selected tissue samples from lungs, liver, spleen, lymph nodes, cerebrum, cerebellum, intestines and heart were fixed in a 10% solution of formalin before being routinely processed, sectioned at 5 μm and stained with hematoxylin and eosin (H-E) for light microscopic examination. Selected sections were stained with Masson's trichrome stain, periodic Acid Shift (PAS) and luxol fast blue (LFB).

Immunohistochemical staining

For the detection of viral antigens, the positive control serum of an indirect monophasic ELISA based on the whole-virus antigen (ID Screen MVV/CAEV indirect screening test, IDVet Innovative Diagnostics, France) was used.

Avidin-biotin complex (ABC) method was performed as described previously [12]. Briefly; paraffin sections of 4 μm thickness were deparaffinized in xylene and rehydrated through graded alcohol. The sections were placed in citrate buffer saline (pH 6.0) in a microwave oven for 20 min for antigen retrieval. Endogenous peroxidase activity was blocked with 5.0% H_2O_2 in methanol for 10 min. Primary antibodies were diluted to 1/200. After incubation with primary antibodies immunodetection for 60 min at 37°C was performed with anti–polyvalent, followed by peroxidase-labeled streptavidin using a labeled streptavidin biotin kit with a 3-Amino-9-Ethylcarbazol (AEC) (Thermo Scientific), as chromogen as the substrate. Sections were counterstained with hematoxylin, and coverslips were attached using aqueous mounting media.

Polymerase chain reaction assays

Proviral DNA Extraction: The DNA was extracted from tissues and blood samples by using GF-1 Viral Nucleic Acid kit (Cat: GF-TD-100, Vivantis, Malaysia). The primers were used as described earlier [13]. They were used for Long Terminal Repeat (LTR) gene of SRLV (CAEV). The PCR was optimised in 30 μl reaction volume inclueded 1xTaq DNA polimerase buffer, 2.4 mM $MgCl_2$, 10 pmol each forward and reverse primer, 100 μM dNTP mix 0.5 IU Taq DNA polimerase (Thermo Scientific, USA) and 3 μl DNA templete. Amplification was carried out for 35 cycles each at 94°C for 1 min, 65°C for 1 min and 72°C for 1 min with final extension at 72°C in a thermal cycler (Verity, Applied Biosystem, USA). The PCR was analysed in a 1.0% agarose gel. The amplified product of 291 bp was accepted as positive result for CAEV as described earlier [13].

RESULTS

Signalment and history

The history revealed that 50 of 700 animals showed the clinical findings begining 2 months earlier. These signs included anorexia, mastitis, swelling in joints, lameness, dyspnea, head pressing and death. As the disease progressed, some goats were moving on their knees and were unable to stand. The age of goats averaged in 2-3 years, whereas kids were 2-6 months-old. As the condition had previously been diagnosed tentatively as mycoplasmosis by a private practioner, the animals were injected antibiotics (enrofloxacin) against mycoplasmosis without improvement.

Clinical Data

Clinical findings included hair loss, pneumonia, arthritis in one or more joints, while the kids showed depression, weakness, pneumonia, ataxia and paresis or paralysis. A presumptive diagnosis was CAEV infection on the basis of the history and clinical findings.

Gross Findings

Necropsy revealed 3 of the animals were markedly cachectic. There was a decrease in body fat and serous atrophy in the coronary band. The lungs were collapsed and firm on palpation. On cut sections, they were gelatinous and bulged. No signs of arthritis or synovitis were present in the animals.

Histologic Lesions

The main histologic lesions are presented in Table 1.

Table 1. Severity and distribution of major histopathological findings in goats.

Case Number	Interstitial Pneumonia	Alveolar Proteinosis	Demyelination	Gliosis	Interstitial Nephritis	Focal interstitial myocarditis
1	+	+	+	++	+	-
2	++	++	++	+	++	++
3	+++	++	++	++	++	-
4	+	+	-	-	-	-
5	+	+	-	+	+	+
6	++	+++	++	+	-	++

Lungs: Mild to moderate interstitial pneumonia was the most prominent lesion in all animals. Thickening of alveolar septums was due to proliferation of alveolar type II epithelial cells and infiltrations of mononuclear cells, mostly macrophages (Fig-1A). Alveolar spaces were filled with excessive acidophilic and protein-like (lipoprotein-

like) material (Fig-1B). The other remarkable lesion is mild to moderate smooth muscle hypertrophy in the wall of terminal bronchioles and alveolar ducts (Fig-1C). Hypertrophy of smooth muscles and distortion of the air spaces (honeycomb appearance) were detected. Interstitial fibrosis was mild to minimal. Alveolar histiocytosis was present in 3 cases and multifocal microabscesses with intralesional

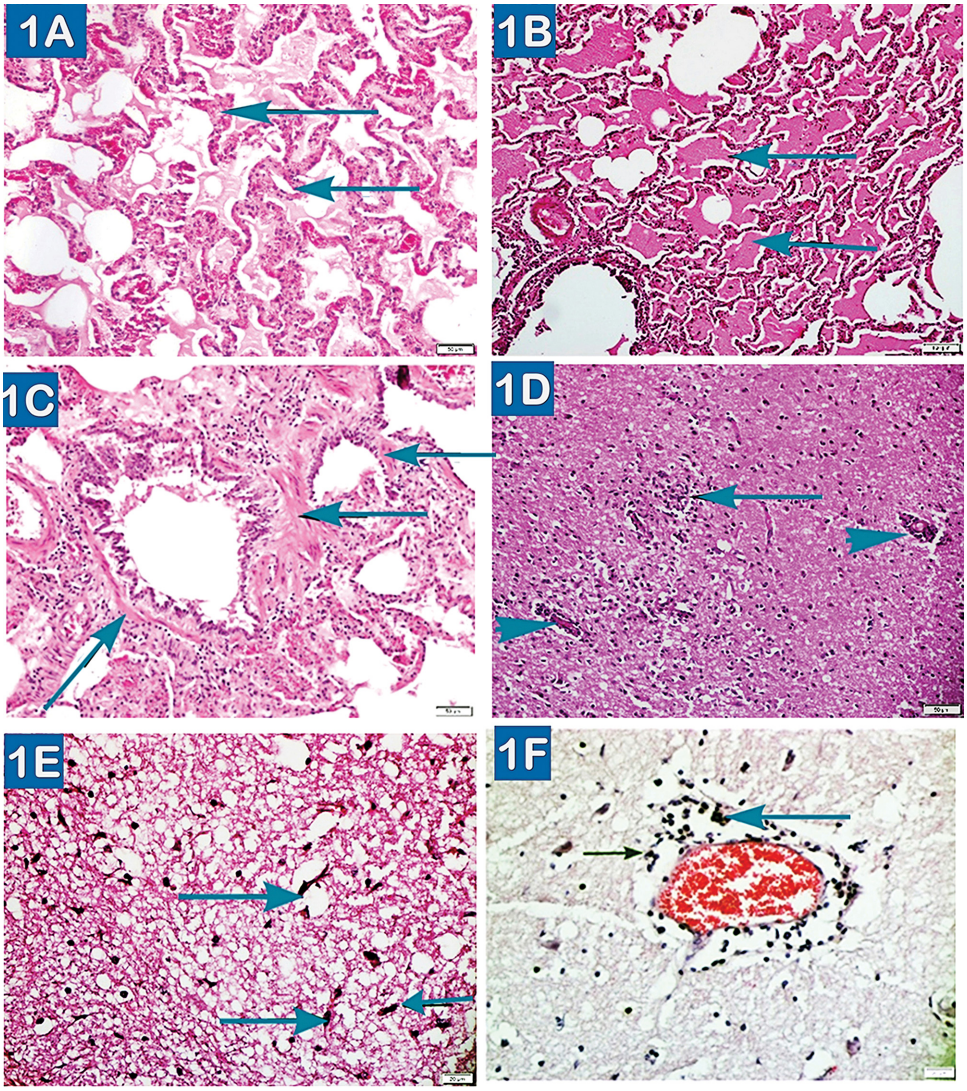

Figure 1. (A) Thickening of interalveolar septum (arrows), due to chronic lymphocytic interstitial pneumonia H-E, x20. **(B)** Intraalveolar eosinophilic proteinaceous fluid (arrows) and expansion of alveolar septa, H-E, x20. **(C)** Muscular hypertophy around the bronchioles (arrows), H-E, x20. **(D)** Microglial nodule (arrow), vascular proliferation (arrow heads) and reactive vascular endothelial cells in white matter, medulla oblangata, H-E, x40. **(E)** Reactive glial cells in white matter of pons, H-E, x40. (**F)** Lymphocytic cell infiltration in the Virchow-Robin space (arrows), H-E, x40.

bacterial colonies were present in 2 cases. A varying degree of peribronchial and perivascular lymphohistiocytic infiltration and locally extensive emphysema and atelectasis were the other findings detected.

Cerebral and Cerebellar Lesions: Moderate to severe demyelinisation, perivascular cuffing, gliosis, capillary vascular proliferations were present in the white matter (Fig1-D) of the cerebrum and cerebellum. There was a loss of neuropil and numerous gitter cells. Gliosis was characterized by elongation and hypertrophy of glial cells in some areas (Fig1-E).

Myelin sheaths were multifocally lost, dilated and empty. Submeningeal white matter and paraventricular regions were the most markedly affected. Virchow-Robin spaces were expanded by mononuclear inflammatory infiltrate (Fig-1F). In 2 cases, the meninges are focally infiltrated with lymphohistiocytic infiltrate. Adjacent capillaries contained reactive endothelium.

Cardiac Lesions: Mild to moderate interstitial edema, focal myocardial necrosis and lymphohistiocytic infiltration were detected (Fig-2A) in some animals.

Renal Lesions: Mild to moderate interstitial nephritis and interstitial fibrosis were present in 4 of 6 goats. There was also marked perivascular lymphohistiocytic infiltrates in the medullar vessels. Other lesions included hyaline cylinders and focal interstitial hemorrhage.

In all cases, hepatic atrophy was detected characterized by small hepatocytes with reduced cytoplasm. Due to the shrinkage of the acini, the portal spaces and vena centralis were closer to each other.

Immunohistochemical analysis

Viral antigens were detected in glial cells, ependymal cells, alveolar macrophages, perivascular renal infiltrate and tubular epithelial cells.

PCR analysis

PCR was performed on both tissue samples and blood samples collected in herd screening. The total number of PCR samples was 24. In 5 of these samples, a product of 291 bp size was detected by PCR (Fig-2C).

ELISA

As a result of the ELISA test performed for herd screening, 3 positive animals, 1 of which was positive and 2 of which were in the suspicious positive range, were detected from 10 samples.

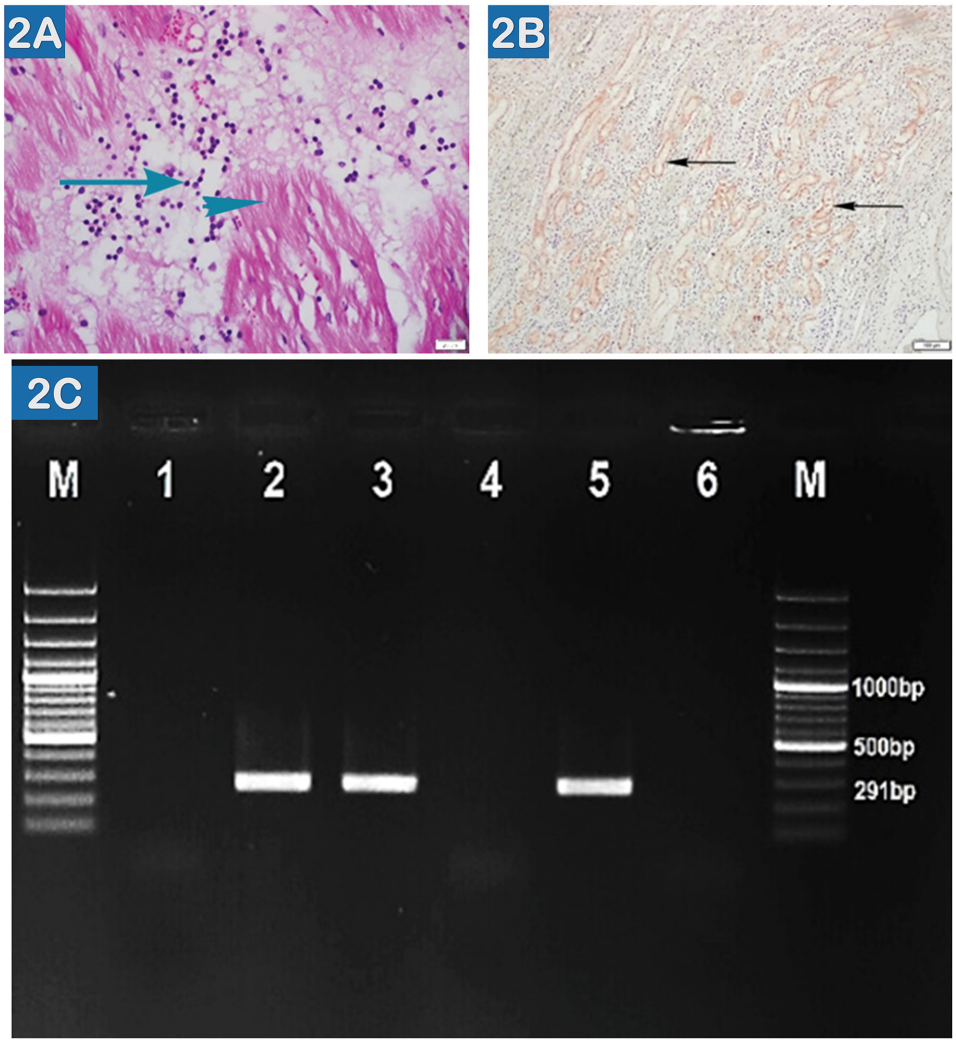

Figure 2. (A) Focal myocardial necrosis (arrow head) and lymphohistiocytic infiltration (arrow) in heart, H-E, x40. **(B)** Antigen positive cells in the tubular epithelium (arrows), ABC immunoperoxidase method, x20. **(C)** PCR result using the CAEV LTR gene region forward and reverse primer pairs (for 291bp LTR). M: DNA ladder of 100 bp (Thermo scientific, USA) Lane 1: Negative control (Water), Lane 2: Positive control, Lanes 3 and 5: Positive field sample, Lanes 4 and 6: Negative field sample.

DISCUSSION

We are reporting clinical, pathological and immunohistochemical and molecular findings of CAEV infection, the pathogen causing latent infections, rare premortal findings in goats and sheep. Clinical and histopathologic diagnosis was confirmed using PCR and immunohistochemistry.

CAE is a multisystemic chronic disease causing great economic losses in goat breeding in terms of decreased milk production, birth weight, live weight gain and high mortality rate. However, no gold standard method is available for CAEV diagnosis. Despite to this disadvantage, the combination of serological and molecular techniques might be optimal detection for CAEV infection [10].

The exact morbidity of CAEV infection is unknown due to delayed seroconversion or intermittent antibodies and the genetic heterogeneity of regional virus strains. In support of this view, using ELISA analysis, only 3 out of 10 goats having clinical signs were diagnosed positive or suspected in this study. However, they were all positive in PCR examination. PCR and isothermal amplification methods appear to be more suitable to detect infected animals.

Clinically, all findings of CAEV infection were detected in the present report. Histopathologically; chronic pneumonia due to caprine arthritis-encephalitis has 2 distinct features not seen other small ruminant viral pneumonias. These are intraalveolar eosinophilic proteinaceous fluid accumulation and smooth muscle hyperplasia in the walls of the alveolar ducts and terminal bronchioles [1,3].

In addition to classical lesions, focal myocarditis and interstitial nephritis were detected in some animals. The presence of viral antigen in renal tubular epithelium confirms that kidney lesions may be virus-induced in the present study. However, a recent study showed the presence of viral antigen in arterial tunica media and tubular epithelium in the renal medulla [14].

Authors' contributions

MOT carried out the molecular studies, participated in the sequence alignment, carried out the immunoassays and drafted the manuscript. EB carried out the clinical examination and carried out the immunoassays. YE, AC, BK and CAI carried out necropsy and histopathological examination. YE and HE participated in the design of the study and coordination and helped to draft the manuscript. All authors read and approved the final manuscript.

Declaration of conflicting interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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KLINIČKI, PATOLOŠKI I MOLEKULARNI NALAZI TOKOM INFEKCIJE VIRUSOM ARTRITISA I ENCEFALITISA KOD DAMASCUS KOZA

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U radu su prikazani rezultati kliničkih, patoloških i molekularnih ispitivanja kod muznih koza inficiranih virusom izazivačem artritisa i encefalitisa koza, u stadu u Turskoj. Prema anamnezi, 50 od ukupno 700 koza i jarića, pokazivalo je kliničke simptome uključujući anoreksiju, mastitis, otok zglobova, šepanje, dispneju i guranje glave, u periodu od dva meseca. Petnaest životinja je ispitano kliničkim pregledom a kod 6 koza je obavljena i analiza patoloških promena. Klinički, uočena je depresija, slabost, pneumonija i ataksija i pareza kod 15 koza. Histološkom analizom, ustanovljene su promene u vidu intersticijalne pneumonije različitog intenziteta, nesupurativnog leukoencefalomijelitisa, intersticijanog nefritisa i intersticijalnog miokarditisa. Imunohistohemijskim metodama, dokazano je prisustvo virusnog antigena u tubularnom epitelu, u glija ćelijama i u makrofagima. Može se zaključiti da se radi o prvoj prijavi i dokumentovanim patološkim i molekularnim promenama karakterističnim za infekciju virusom artritisa i encefalitisa koza u Turskoj.