



Ovine adenoviruses infecting sheep and goats in Türkiye: detection and molecular characterization of three different types

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Abstract

Adenoviruses (AdVs) have been detected in a wide variety of animals. To date, eight types of AdVs in sheep and two types in goats have been identified, which belong to two distinct genera, *Mastadenovirus* and *Atadenovirus*. Typically, the term pneumo-enteritis is used to describe adenovirus-induced disease in small ruminants, which has been associated with both enteric and respiratory symptoms of varying severity. The aim of this study was to detect and identify AdVs of small ruminants belonging to the genera *Mastadenovirus* and *Atadenovirus*. For this purpose, diagnostic samples (47 lung, 27 intestine, and two pooled tissue samples including intestine and lung) from 49 small ruminants (39 sheep and 10 goats) were used. Following the viral DNA extraction, PCR was carried out by using the primers targeting the hexon gene in order to detect both mast- and atadenoviruses. Sequencing the amplified fragments revealed the presence of three types of ovine adenovirus (OAdV): OAdV-3, OAdV-4, and OAdV-8. Specifically, OAdV-3 was detected in two sheep and a goat while OAdV-4 and OAdV-8 were found in only one sheep each. There is still limited data on the interaction between the viruses in different adenovirus genera and the detected disease, as well as the genetic diversity of adenoviruses, especially in small ruminants. In conclusion, the detection of AdVs in lung and intestinal tissues of small ruminants in this study suggests that these viruses may have contributed to the disease and/or predisposed to other agents.

Keywords Adenovirus · OAdV · Sheep · Goat · Hexon gene · Type

Adenoviruses are non-enveloped double-stranded DNA viruses. They have icosahedral capsid possessing 240 hexon proteins, which are the primary component of the capsid (919–968 amino acids), and 12 fiber attachment proteins linked to 12 penton base proteins involved in recognition and interaction with cellular receptors [1]. Specific antigenic determinants located in the hexon proteins of adenoviruses are the basis for the variation in the serological cross-reactivity of adenoviruses [2]. So far,

eight types of AdVs from sheep and two types of AdVs from goats have been identified, according to the International Committee on Taxonomy of Viruses (ICTV) [1]. Small ruminant AdVs in *Mastadenovirus* belong to three species: *Ovine mastadenovirus A*, *Ovine mastadenovirus B* and *Ovine mastadenovirus C*. *Ovine mastadenovirus A* consists of ovine adenoviruses 2–5 (OAdV-2-5) whereas *Ovine mastadenovirus B* is composed of OAdV-1 in addition to caprine adenovirus 2 (GAdV-2) [1, 3]. A recently identified distinct type of ovine mastadenovirus, OAdV-8, is the only member of *Ovine mastadenovirus C* [4]. According to the 9th report of ICTV, OAdV-6 is defined as a virus that may be a member of the genus *Mastadenovirus* but has not been approved as species [3]. The species *Ovine atadenovirus D*, which includes types OAdV-7 and GAdV-1, is located in the genus *Atadenovirus* [1]. Adenoviruses have been associated with clinical disease in sheep and goats [5–9]. AdVs have been isolated from sheep and goats that appeared to be healthy, as well as from those exhibiting signs of disease in the respiratory and digestive systems [4–12]. Unfortunately, little is

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known about the pathogenic functions of sheep and goat adenoviruses and their molecular epidemiology in relation to various diseases. Therefore, it was aimed to investigate and molecularly characterize the hexon gene of the detected AdVs in the samples from small ruminants with pneumoenteritis symptoms in this study.

In this study, lung ($n=47$), intestine ($n=27$), and pooled tissue samples (lung and intestine) ($n=2$) obtained from a total of 49 small ruminants (39 sheep and 10 goats) with pneumoenteritis from different farms in several provinces of Türkiye were used.

Samples were screened for mastadenovirus (MaAdV) and atadenovirus (AtAdV) by PCR. The samples were homogenized with phosphate-buffered saline (PBS) at a ratio of 1:10 (w/v). Viral DNA was purified with the QIAamp DNA Mini Kit (Qiagen, Germany) and stored at -80°C . For the detection of mast- and atadenoviruses, a semi-nested PCR technique was applied using the primer sets targeting the hexon gene, containing type-specific epitopes [13]. Briefly, the PCR mixture consisted of 3 μl of 10 $\times$ Taq buffer, 2.4 μl of MgCl_2 (25 mM), 1.25 μl (10 pmol/ μl) of each primer, 1 μl of dNTP (10 mM each), 0.25 μl of Taq polymerase (500 U/ μl) (Thermo Fisher Scientific, USA), and 3 μl of target DNA and nuclease-free water added up to 30 μl of the final volume. In the second round, 3 μl from the reaction mixture of the first round was used as a template. The optimized PCR thermal conditions were performed for both rounds as follows: denaturation at 94°C for 4 min 30 s, 35 cycles of denaturation at 94°C for 30 s, annealing at 55°C for 30 s, extension at 72°C for 50 s (with an increment of 1 s per cycle), and final extension at 72°C for 7 min. The amplification products were run in 1% agarose gel stained with SafeView Classic (ABM, Canada), and visualized under UV light. The mastadenovirus (OL513130) detected in our previous study [14] was used as a positive control for mastadenovirus in this study. In addition, a positive control for atadenovirus was kindly provided by Prof. Dr. M. Ozkan Timurkan (Atatürk University).

The second-round PCR products with the expected size were purified and sequenced using bidirectional Sanger dideoxy sequencing. Multiple sequence alignments were prepared by the MUSCLE algorithm as implemented in Aliview Software [15, 16]. Cognate

sequences of reference AdVs representing different types were retrieved from GenBank through the BLAST engine. Phylogenetic analyses were conducted using MEGA X software by applying the neighbor joining method [17]. Before applying the method, the “Find Best DNA/Protein Model” feature of MEGA X software was used to choose the best fit model. The nucleotide substitution model was selected according to Bayesian information criterion (BIC). Accordingly, the phylogenetic trees were constructed using the Kimura 2 + G nucleotide substitution model. The nucleotide (nt) and amino acid (aa) identities were calculated using the SIAS on-line tool (<http://imed.med.ucm.es/Tools/sias.html>). The sequences detected in this study were submitted to the GenBank under the following accession numbers: OQ572348–OQ572351 and OR348861–OR348863.

Besides causing subclinical infections, AdVs are considered one of the important pathogens causing respiratory and intestinal disease in ruminant species [4–12, 18–20]. However, there is limited data regarding both seroprevalence and detection of AdVs, particularly in small ruminants. Our results revealed that out of 49 small ruminants, five (10.2%) from different farms ($n=5$) in three provinces (Krac14 and Krac24 from Eskisehir, Krac18 and Krum50 from Ankara, and Krac25 from Bartın) were found positive for the hexon gene of MaAdV. However, no amplicons were yielded using the AtAdV hexon gene-specific primers. While four of the MaAdV-positive samples were obtained from sheep (4/39, 10.2%), one was obtained from a goat (1/10, 10%). The AdVs have been isolated from apparently healthy lambs, but they have also been found in animals with severe pneumonia and enteritis [19–22]. This study focused on AdVs in both lung tissues and intestines of small ruminants. In detail, among all MaAdV-positive animals, only intestinal samples of Krac25 were not available. For two of the MaAdV-positive animals (Krac18 and Krac24) both lung and intestinal samples were positive, whereas interestingly only the lung sample of Krac14 was positive. The sample of Krum50 was the pooled intestine and lung tissues (Table 1). The results suggest that besides gastroenteritis these viruses may have contributed to the respiratory disease and/or may predispose to other respiratory tract agents in these animals in our country.

To date, only a few studies have reported AdVs in small ruminants and their molecular characterizations [2, 4]. The molecular and phylogenetic analyses of a portion

Table 1 Detailed information of MaAdVs detected in this study

Animals	Species	Province	Sample type/Acc. no.		MaAdV
			Lung	Intestine	
Krac14	Sheep	Eskisehir	OQ572348	Negative	OAdV-3
Krac18	Goat	Ankara	OQ572349	OR348861	OAdV-3
Krac24	Sheep	Eskisehir	OQ572350	OR348862	OAdV-3
Krac25	Sheep	Bartın	OQ572351	NA	OAdV-4
Krum50	Sheep	Ankara	OR348863*		OAdV-8

NA Not available

*Pooled sample

of the hexon gene region (for ~565 bp) of the MaAdVs in the current study characterized three types of OAdV: OAdV-3, OAdV-4, and OAdV-8. Specifically, OAdV-3 was found in two sheep (Krac14, and Krac24) and a goat (Krac18) while OAdV-4 was found in just a single sheep (Krac25). Furthermore, OAdV-8 was identified in a sheep (Krum50). As expected, the OAdV-3 sequences obtained from the lung and intestinal samples of Krac18 and Krac24 were identical (100%) for each of the respective samples. The three Turkish OAdV-3 strains shared 98.76–100% nt and 99.47–100% aa sequence identity with each other. They displayed 97–97.88% nt and 99.47–100% aa sequence identity with the prototype strain OAdV-3/PX611/IRL. Furthermore, it should be stated that this is the first report of OAdV-3 in a goat. The other MaAdV classified as OAdV-4 in this study shared 99.82% nt and 99.46% aa sequence identity to OAdV-4/7769/IRL strain. Finally, it was revealed that the sequence of the amplified fragment of Krum50 was related to the prototype OAdV-8/7508/HUN strain with 93.99% nt identity. The partial hexon gene regions of the Krum50 strain were similar to this strain, with 94.84% aa identity. Phylogenetic analysis based on the partial hexon gene region (Fig. 1) confirmed the presence of three types of OAdVs. Accordingly, OAdV-3 strains, Krac14, Krac18, and Krac24 grouped with the reference OAdV-3/PX611/IRL strain while Krac25 shared the same branch with the reference OAdV-4/7769/IRL strain. Lastly, Krum50 was clustered with the newly detected OAdV-8/7508/HUN strain.

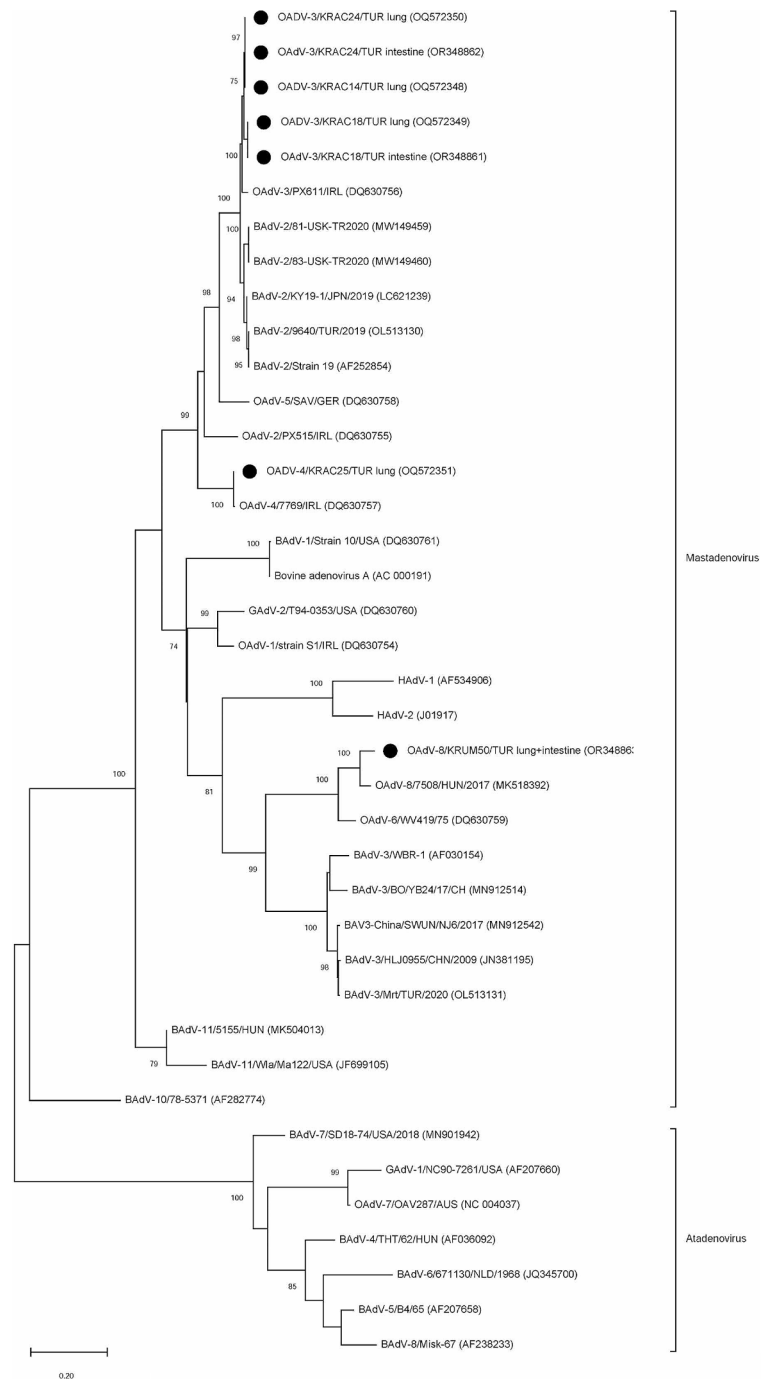
Seroprevalence studies conducted in Türkiye a long time ago demonstrated a significant seroprevalence to a variety of distinct AdV serotypes in both sheep and goats. Öztürk [23] found neutralizing antibodies against OAdV-5 at the rate of 22.29% in the sera of 700 sheep obtained from various regions in Türkiye. Burgu et al. [24] detected the seroprevalence rates for OAdV-1 at 38.2%, for OAdV-3 at 19.2%, and for OAdV-5 at 21.2% in sheep. In another study, similar rates were also detected in sheep: 29.56% and 33.04% for OAdV-1 and OAdV-3, respectively [25]. In goats, the seroprevalence rates were 4.4% for OAdV-1, 4.8% for OAdV-2, 9.2% for OAdV-3, and 7.2% for OAdV-5 [26]. These studies revealed that adenoviruses are prevalent in sheep and goats in our country. Nevertheless, data on the detection of adenoviruses in sheep and goats in our country are very limited. Previous reports showed that OAdV-3 and OAdV-5 had been isolated from fecal samples from sheep [20, 27]. Also, AdVs have been detected in the intestinal tissues of lambs and kids by immunohistochemical (IHC) analysis [8]. In addition, AdV positivity rates of 19.3%, 22.7%, 20.4%, and 13.6% were found in lung samples collected from sheep with pneumonia using IHC, IF,

ELISA, and PCR, respectively [21]. However, no molecular characterization data of these viruses from small ruminants have been reported yet. In fact, global data on this subject is extremely scarce. Since the first detection of OAdVs in 1969 [22] and GAdV-1 in 1999 [11], the data on the genetic diversity of AdVs in small ruminants is still limited. AdVs in sheep and goats have not been isolated or detected by PCR for a long time, except for the recently discovered unique OAdV-8 mastadenovirus species [4]. Generally, partial genome sequences of the known OAdVs belonging to the genus *Mastadenovirus*, mostly from the hexon gene, are available in the GenBank database. Only one hexon gene sequence for each strain of OAdV-3 (DQ630756) and OAdV-4 (DQ630757) and the complete genome sequence for OAdV-8 has so far been deposited. This study presents significant findings on the molecular identification of three distinct types of ovine adenoviruses (OAdV-3, OAdV-4, and OAdV-8) in sheep and goats. The results contribute valuable data to GenBank and the existing literature and enhance our understanding of adenoviruses that are prevalent in these particular animal species in Türkiye.

We also report an OAdV-8 strain identified in a lamb (Krum50) that died from pneumoenteritis. The first OAdV-8 was detected in a suckling male lamb showing severe viral pneumonia characteristic of adenoviral infection with suspected bacterial superinfection in Hungary, in 2017 [4]. For our strain, only the partial hexon gene sequence is available in this study and this region showed 94.84% aa sequence identity to the corresponding region of OAdV-8 (MK518392). Considering that OAdV-8 is a recently identified type, it is particularly suggested that more detailed molecular studies should be conducted. Thus we planned further studies to obtain relevant information on the complete genome sequence of OAdV-8 or at least some gene regions (e.g. pol gene, fiber), just as it was planned for OAdV-3 and OAdV-4.

In conclusion, we report the detection of several OAdVs, including type 8, in samples of small ruminants that died due to acute or chronic pneumoenteric disease in Türkiye. Our results indicate that different OAdVs circulating among small ruminants in Türkiye might be involved in severe diseases. This study provides insight into the detection and molecular epidemiology of OAdVs in small ruminants in our country. Since there still remains much to be known about OAdVs, further studies are needed for active molecular epidemiological surveillance of OAdVs to determine their prevalence and clinical significance for small ruminants.

Fig. 1 Phylogenetic tree of the nucleotide sequences of the hexon gene of OAdV strains identified in this study with those of other MaAdVs and AtAdVs selected in GenBank. According to the alignment of nucleotide sequences of the hexon gene (~565 nt) the phylogenetic tree was generated using the neighbor joining method provided by MEGA X software. Phylogenetic distances were statistically supported by bootstrapping with 1000 replicates. The positions of Turkish OAdV strains are indicated by a black dot. Bootstrap values < 70% are not shown. The scale bar indicates nucleotide substitutions per site. The genera are indicated on the right-hand side of the phylogenetic tree



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Data availability The data that support the findings of this study are available from the corresponding author upon reasonable request.

Declarations

Ethical approval No ethical approval was required for this study. In this study, retrospective samples sent to our laboratory by field veterinarians for diagnostic purposes were used.

Competing interests The authors declare no competing interests.

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