

A retrospective investigation of canine adenovirus (CAV) infection in adult dogs in Turkey

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ABSTRACT

Canine adenovirus (CAV) type 1 and 2, respectively, cause infectious canine hepatitis and infectious canine laryngotracheitis in members of the families *Canidae* and *Ursidae* worldwide. Both of these infections are acute diseases, especially in young dogs. The aim of this study was to conduct a serological investigation of canine adenovirus infection. For this purpose, serum samples were collected from native pure-bred Kangal ($n = 11$), and Akbash dogs ($n = 17$) and Turkish Greyhounds ($n = 15$) in Eskişehir and Konya provinces. None of the dogs were previously vaccinated against CAV types. Indirect ELISA detected 88.2 %, 93.3 % and 100 % prevalences in Akbash, Greyhound and Kangal dogs, respectively. The remainder of the samples ($n = 51$) were collected at the Afyonkarahisar Municipality Shelter. Forty-two of these dogs (82.3 %) were detected as seropositive. In total, 82 of 94 dogs (87.2 %) were found to be positive for CAV serum antibodies.

Keywords: antibody, canine adenovirus, dog, Turkey.

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INTRODUCTION

Canine adenovirus (CAV) type 1 and 2 are respectively responsible for a systemic disease and respiratory tract infection in both domestic and wild canid species^{1,20}. Although there is antigenic relatedness and cross-protective immunity between the 2 types¹³, restriction endonuclease analysis shows that they are genetically different viruses². Adenovirus virions have non-enveloped icosahedral symmetry. The genome consists of a linear molecule of double stranded DNA, 36–44 kbp in size, with inverted terminal repeats. Nearly 40 proteins are encoded by the viral genome, one-third of which are structural proteins¹⁶.

CAV-1 causes infectious canine hepatitis (ICH), recognised by Rubarth in 1947 as a specific viral disease of dogs²⁰. It is characterised by asymptomatic to fatal disease¹⁵. Virus enters the host *via* direct contact with contaminated saliva, urine and faeces⁶. The incubation period is 4–7 days. The agent has an affinity for endothelium and hepatocytes. The main clinical findings are rhinitis, ataxia, anorexia, tonsillitis, abdominal pain, blood in faeces,

acute/chronic hepatitis and interstitial nephritis. Encephalitis is an infrequent event but when it occurs, death can follow rapidly, with lethargy, ataxia, blindness and vomiting¹⁰. Bilateral opacity of the eyes, referred to as 'blue eye', can be seen in 25 % of the affected animals 7–10 days after the resolution of the acute clinical signs, and is due to corneal oedema and accumulation of antigen-antibody complexes in the anterior chamber^{7,8,23}.

CAV-2 was first detected in 1961 in dogs with clinical signs of laryngotracheitis by Ditchfield and co-workers, who named it infectious canine laryngotracheitis (ICLT)¹². CAV-2 is a virus that causes respiratory disease, with clinical signs that include tonsillitis, pharyngitis, tracheitis and bronchitis. As a result of infection by this disease, bronchiolitis obliterans can become permanent in recovered puppies⁹.

Both of the infections generally have a good prognosis, being totally silent in adults, although neonates and juveniles are more sensitive. Both of the agents cause respiratory disorders, but CAV-2 is associated with more serious disease, including severe fatal bronchopneumonia^{3,22}. The clinical course of CAV-1 infection can range from peracutely fatal without clinical signs, acutely fatal with respiratory signs in pups, to mild disease resulting in resolution of clinical signs. The acute form can also be fatal with typical findings. Mild disease generally

results in full recovery¹⁶.

Colostrum antibodies will confer protection until their immune systems are more mature, and thus maternal immunity against CAV-1 and 2 is an important component of neonatal protection^{4,13}.

Kangal, Akbash (Akbaş) and Turkish Greyhound dogs are native breeds of Anatolia^{14,17}. The Kangal is a shepherd dog with a characteristic temperament and morphology, mostly reared in central Anatolia. The Akbash has been bred as a shepherding and guard dog in central-western Anatolia. The Turkish Greyhound is a distinct Greyhound breed throughout Anatolia, mostly in the central and southern region, but today the population is very small. There are limited studies on neonatal deaths attributable to CAV infections in these breeds. Immunisation programmes have not been properly instituted in rural areas in Turkey. Ensuring maternal protection is the 1st step in a preventative programme, as puppies of seropositive bitches may be protected until any time from 9 weeks to 4 months of age. As far as the authors are aware there has been only 1 serological study that has investigated ICH in dogs in Turkey, in 1975, reporting a high incidence¹⁹ of the disease.

The objective of this study was to determine the prevalence of CAV types 1 and 2 exposure in individually reared and shelter-housed dogs in Turkey.

MATERIALS AND METHODS

Selection of animals for sampling

This study was conducted in apparently healthy adult (1 year old and above) dogs in central and western Anatolia. Blood samples were obtained from a newly established shelter in Afyon, and from privately owned pure-bred dogs from 43 different enterprises that rear domestic ruminants in the rural area in Eskişehir and Konya provinces (Fig. 1). Blood samples were obtained from pure-bred adult Akbash ($n = 17$), Turkish Greyhound ($n = 15$) and Kangal ($n = 11$) dogs living on small farms in Eskişehir and Konya provinces (central Anatolia). The number of dogs housed per farm varied from 1 to 6 and were all unvaccinated

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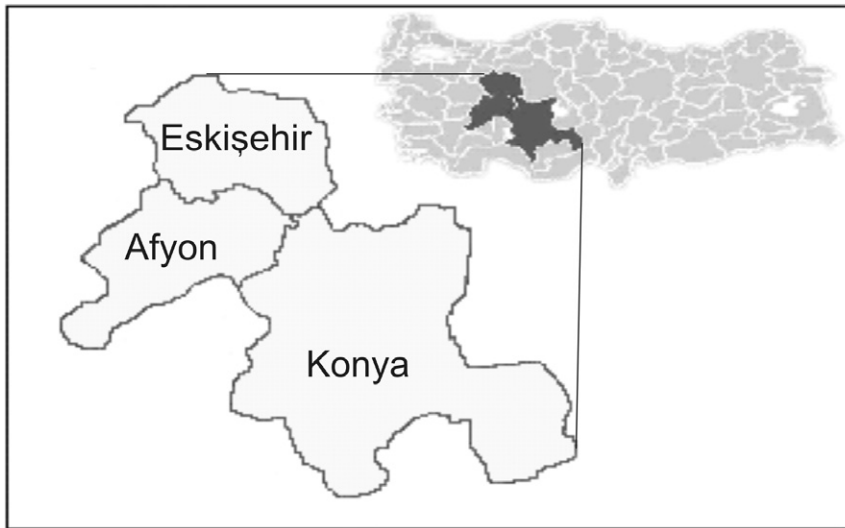


Fig. 1: Map of Turkey (study area in dark grey) showing the location of provinces where dogs were sampled for antibodies against canine adenovirus (CAV).

(except for the Kangal dogs from the Konya provinces that may have received vaccinations, some of them as puppies, but only unvaccinated individuals and individuals of known vaccination status were sampled). A single dog was sampled from each sampling unit as a representative of each group's exposure. Bona fide health records of the sampled dogs indicating whether they had shown clinical signs referable to CAV previously were not obtained.

The remainder of the samples ($n = 51$) were collected from dogs housed in the recently established Afyon Municipality Dog Shelter where animals had been admitted during the 3 months preceding the sampling date. Fifty-one of the 70 shelter housed dogs were sampled, all of them adult dogs (≥ 1 yr).

Management conditions in the shelter were considered optimal, with no more than 2 dogs in adjacent half-enclosed cages. The dogs housed in the shelter in Afyon province had unknown vaccination status prior to admission, but vaccination was considered unlikely. All dogs had received only a rabies vaccination on arrival at the shelter. Sex of the dogs was ignored during sampling. A total of 94 serum samples was collected from adult and clinically normal dogs from breeding units and the animal shelter.

Blood was collected from the *vena saphena* into serum tubes and centrifuged at $3000 \times g$ for 10 minutes. Samples were frozen (-20°C) until analysis.

ELISA (Enzyme-Linked Immunosorbent Assay)

Canine adenovirus indirect ELISA Kit (EVL diagnostic division, Woerden, Netherlands) was used for detecting CAV specific polyclonal antibodies qualitatively in dogs¹⁸. This test is unable to

discriminate between the 2 serotypes. The test was performed in 96-well microtitre plates according to the manufacturer's instructions, and the optical density was measured at 450 nm. Every sample was scored using control values.

RESULTS

Using the I-ELISA test, antibody prevalences of 88.2%, 93.3%, and 100% respectively were detected in Akbash, Greyhound, and Kangal breed dogs housed on rural farms. The lowest prevalence (82.3%) was detected in shelter dogs in Afyonkarahisar province. The highest prevalence was found in Konya Province, with a level of 96.1% (25/26) (Table 1).

DISCUSSION

The privately owned dogs (Kangal, Akbash and Turkish Greyhound) were working dogs, used for shepherding or guarding, and were from the rural area of Eskişehir and Konya. All the samples from the Kangal breed of dogs in Konya province were found to be positive. According to the owners, some of these dogs had received vaccinations, including CAV, as puppies. Autoimmunisation may have occurred in non-vaccinated dogs when those dogs that had received modified live vaccine shed virus, thus exposing unvaccinated dogs. This appar-

ently only occurred in Kangal dogs. Samples collected in Eskişehir province were from Akbash dogs and Greyhounds. Of 17 Akbash dogs, 15 (88.2%) were found to be positive. Of the Turkish Greyhounds, a rare breed, 15 dogs were sampled and 14 of them (93.3%) were seropositive for CAV.

Shelter dogs, without a CAV vaccination history, were from Afyonkarahisar Municipality. Samples were collected just 3 months after the shelter was established when the premises were unlikely to have had a high viral load. The lowest prevalence of antibodies was determined for the animals housed in this shelter (82.3%). It is known that the incidence of an infection with a high morbidity rate, such as CAV, is generally high in dog barns and shelters, making the results of this study unexpected. Possible explanations for the low proportions detected include length of time the dogs were housed in the shelter, samples were collected within only 3 months of its opening, and the difference in management and hygiene in the shelter compared with that of privately owned working dogs. Breed population differences with regard to variable susceptibility could be another compounding factor.

Canine adenovirus has a worldwide distribution. Antibody prevalences that have been reported ranged from 30% to 82% in the dogs tested^{4,20,21}. The prevalence of ICH has been reported to be high in Turkey¹⁹.

Adenoviruses are very resistant to aversive environmental conditions, as well as to chemicals such as chloroform, ether, acids and formalin⁵, and they can survive for long periods. Recovered dogs may shed the virus for a long time. In addition, vaccinated dogs can also shed the virus. These factors lead to a virus burden in the environment.

Clinical cases of ICH have not been seen in regions where vaccination programmes have been in place for several years, possibly due to secondary immunisation. Vaccination against CAV-1 and 2 has not been widely used in rural areas in Turkey. Except for Kangal dogs from Konya province, the dogs in this study were generally not vaccinated, and except for in Kangal

Table 1: Results of CAV antibody status and proportion of exposed dogs from the Anatolian region of Turkey tabulated according to their province of origin and breed.

Provinces	Breed	No. of samples	CAV	
			Ab (+)	(%)
Afyonkarahisar	Shelter dogs	51	42	82.3
Eskişehir-Sivrihisar	Akbash	17	15	88.2
Konya	Greyhound	15	14	93.3
Konya	Kangal	11	11	100.0
Total		94	82	87.2

dogs as previously mentioned, secondary immunisation has not been a consideration in this study.

It is known that the Afghan hound is particularly susceptible to CAV infection¹¹. The breeds included in this study are native to the Anatolia area, and their susceptibility to CAV or other infections has not been previously reported. Owing to the absence of clinical findings in all cases, and the lack of detailed health records from sampled dogs, inferences regarding previous infection are not possible other than that there is a high viral load in the environment, as indicated by high prevalences per province (82.3 % (42/51), 88.2 % (15/17) and 96.1 % (25/26) in Afyon, Eskişehir and Konya respectively. As expected, the highest prevalence occurred in the province that had recorded vaccinations.

The laboratory diagnosis of CAV is mainly based on virus isolation or serology using an enzyme immunoassay, haemagglutination inhibition or the serum-virus neutralisation test. Indirect ELISA is a sensitive, reliable and fast method for the detection of anti-adenovirus antibody¹⁸. However, the kit used for antibody detection in this study could not discriminate between CAV types 1 and 2. The discriminatory power of the test may be considered redundant as both natural infection and vaccination against either of the 2 types provide cross-protection^{1,6,16}.

Our study shows that exposure to CAV and antibody responses are widespread in the provinces studied. Regular vaccination may be useful, especially early in life after maternal antibodies disappear, such as occurs in the Konya province where most dogs are protected.

In conclusion, the seroprevalence of CAV has been investigated for the first time in Eskişehir and Konya provinces of Turkey, and the prevalence in both privately owned dogs in the field and dogs that are confined to shelters appears to be high. It is possible to say that the puppies will be born with protective immunity for the 1st few weeks of life. Further studies

are necessary to determine prevalence of the virus types.

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