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Research Article / Araştırma Makalesi

Seroepidemiology of Akabane Virus Infection in Honamlı Goat Breed

Honamlı Keçi Irkında Akabane Virus Enfeksiyonunun Seroepidemiyolojisi

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Abstract: The goal of this study was to conduct a seroepidemiological investigation of Akabane Virus (AKAV) in Honamlı goats with pure breed characteristics in the hands of the public in Burdur region. For this purpose, blood was collected from 425 goats bred in the mentioned region, unvaccinated, 6 months and older, male and female clinically healthy and was tested for antibodies against AKAV usingCompetitive Enzyme Linked Immunosorbent Assay (C-ELISA) method. The results showed that 9 goat (2.12%) of the 425 were found antibody positive for AKAV infection. Seropositivity rates were found to be between 6.67% and 0% according to the settlements. The distribution of positivity according to age is 3.66% (3/82) in goats in the 4 age group, 3.70% (3/81) in the goats in the 5 age group, 3.45% (2/58) in the 6-year-old goats, and 3.45% (2/58) in the 7-year-old goats. was determined at a rate of 3.33% (1/30) in goats. It was determined that the difference between the seropositivity rates determined in different age groups, males and females and in the districts was statistically insignificant (*P>0.05*). This study is the first serological study to determine seroprevalence of AKAV infection in Honamlı goat in the Burdur Region of Turkey.

Keywords: Akabane Virus (AKAV), ELISA, Honamlı Goat, Seroprevalence.

Öz: Bu araştırmada, Burdur yöresinde halk elinde bulunan saf ırk özelliğine sahip Honamlı keçilerinde Akabane Virus (AKAV) enfeksiyonunun varlığının/yaygınlığının belirlenmesi amaçlanmıştır. Bu amaçla söz konusu yörede yetiştiriciliği yapılan 6 ay ve üzeri, dişi ve erkek sağlıklı görünüme sahip 425 keçiden kan örneklemesi yapıldı. Toplanan kan numuneleri Enzyme Linked Immunosorbent Assay (ELISA) yöntemiyle AKAV antikorları yönünden kontrol edildi. Test edilen 425 keçi kan serumunun 9'u (%2,12) seropozitif olarak belirlendi. Yerleşim yerlerine göre seropozitiflik oranları %6,67-%0 arasında tespit edildi. Pozitifliğin yaşa göre dağılımı ise 4 yaş grubundaki keçilerde %3,66 (3/82), 5 yaş grubundaki keçilerde %3,70 (3/81), 6 yaş grubundaki keçilerde %3,45 (2/58) ve 7 yaş grubundaki keçilerde de %3,33 (1/30) oranında belirlendi. Farklı yaş gruplarında, erkek ve dişilerde ve ilçeler de belirlenen seropozitiflik oranları arasındaki farklılığın istatistik olarak önemsiz (*P>0,05*) olduğu tespit edildi. Bu araştırma sonucunda ilk defa Honamlı keçi ırkında AKAV enfeksiyonunun varlığı ve yaygınlığı serolojik olarak ortaya konulmuştur.

Anahtar Kelimeler: Akabane Virus (AKAV), ELISA, Honamlı Keçi, Seroprevalans.

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Introduction

As one of the important infectious agents in cattles, sheeps and goats, Akabane virus (AKAV) causes various of symptoms including abortion, mummified fetus, stillbirth; blindness, congenital arthrogriposis and hydranencephaly (AH) in offsprings. AKAV is member of *Orthobunyavirus* genus in *Bunyaviridae* family in taxonomy. AKAV is classified in Simbu serogroup which has close relationship with other orthobunyaviruses. The very first agent called OBE-1 was isolated from a naturally infected bovine fetus (Ishihara, 2016) in Japan in 1959 and named after the region (Matsuyama et al. 1960; Kinney and Calisher 1981). Virus carries icosahedral, membraned, 3-segmented ssRNA morphologically (Ludwig, 1991; Sharma ve Adlakha, 2009; Ishıhara, 2016). There are many cell lines developed for the isolation and production of the virus, however, BHK-21, VERO and HmLu-1 cell lines of mammalian origin were used for the production of

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the agent in vitro most of the time. In recent years, insect derivated cell lines are preferred due to detection of more intensive virus reproduction in these cells (Elliot and Blakqori, 2011).

Culicoides spp. biting midges plays the important role in the transmission of the disease and the distribution of these vectors may differ geographically. *Culicoides oxystoma* is common in Japan, *C. brevitarsis* in Australia, *C. milnei* and *C. imicola* is seen in Africa mostly. In addition, other *Culicoides species* seen in North America such as *C. variipennis*, is infected experimentally in laboratory studies. On the other hand, vertical transmission is also important when it comes to epidemiology of Akabane disease. Transmission occurs from non-immune mothers to their offsprings and causes congenital anomalies (Spicler, 2017).

The pathogenesis of Akabane infection differs in pregnant and non-pregnant animals. When AKAV infects pregnant cattle, sheep or goats; it causes various congenital anomalies in the fetus. The incidence and severity of these anomalies depend on the gestational period. This is particularly evident in cattles due to longer gestation period than small ruminants. Abortions, stillbirths and premature births may be the first signs of an Akabane epidemic. Aborted fetuses may appear normal in first examination but disease may be diagnosed with examination of joints. In addition, severe hydranencephaly may be detected if skull is opened (Spicler, 2017). Blindness, nystagmus, deafness, dullness, slow sucking, paralysis and incoordination develop in offsprings born with hydranencephaly. These offsprings can survive for several months if they are fed properly. In adults, infection often occurs subclinically and animals living in endemic areas gain immunity to the disease from early ages (Mellor and Kirkland, 2008).

If clinical symptoms are present and Akabane disease is suspected, final diagnosis should be confirmed by using either serological or virological tests. Enzyme linked immunosorbent assay (ELISA), serum neutralization test (SNT) and as golden standard for diagnosis, polymerase chain reaction (PCR) are used for this purpose (OIE, 2014; OIE, 2016).

In this study, it was aimed to determine the presence of Akabane virus infection serologically by using competitive ELISA method and to obtain information about the prevalence of the Akabane virus in Honamlı goats that unvaccinated, different groups of age and sexin Burdur province. Additionally, due to limited number of studies in Turkey and lack of studies on Honamli goat breed in the world, it was aimed to contribute academic literature of science.

Materials and Methods

Ethics Statement

This research was conducted after the approval of Burdur Mehmet Akif Ersoy University Animal Testing Local Ethics Council (Approval Number: 18.09.2019/547)

Sampled Animals

In this study, blood samples were collected from 425 Honamlı goats that were not immunized against AKAV infection, aged 6 months and older, of different sexes and ages, bred in private small scale family production units in 10 districts (Table 1) of Burdur region (Figure 1).

Table 1. Distribution of collected blood samples by districts and gender

Sampling Districts	Male	Female	Total
Karamanlı/Burdur	18	57	75
Ağlasun/Burdur	2	54	56
Çeltikçi/Burdur	10	45	55
Bucak/Burdur	7	43	50
Tefenni/Burdur	4	21	25
Gölhisar/Burdur	1	23	24
Çavdır/Burdur	14	13	27
Yeşilova/Burdur	13	32	45
Altınyayla/Burdur	1	29	30
Kemer/Burdur	3	35	38
Total	**73**	**352**	**425**

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Figure 1. Political map of Burdur province.

Determining the number of samples to be used in the research; At 99.9% confidence level, 99% confidence interval, 5% margin of error, population size of 30598 (Number of pure Honamlı breed goats in Burdur province: 30598 (2020 TR Ministry of Agriculture and Forestry data) (https://hbs.tarbil.gov.tr) in the herd, considering the other studies conducted in Turkey, the possible prevalence of AKAV infection was calculated as 20%, and the sample size was calculated as 425 (Erganiş, 1993).

Serum Samples

The blood samples used in the study were taken from the vena jugularis into sterile coagulated tubes and brought to the laboratory under cold chain conditions. After the blood samples were centrifuged at 3000-4000 rpm for 20 minutes and then separated serum was transferred to sterile eppendorfs and stored in a deep freezer at -20˚C until the ELISA test.

Competitive Enzyme Linked Immunosorbent Assay (C-ELISA)

The presence of anti-G specific antibodies against the structural G protein of Akabane virus was investigated using C-ELISA in sera obtained from blood samples from Honamlı goats. This method works on the principle of solid phase indirect competitive ELISA. Blood serum samples are placed in the wells of microtiter plates coated with AKA antigen and if AKAV antibodies are present in the serum, they are bound to the antigens. If specific AKAV immunoglobulins are present in the test sera, the conjugate binds to the wells containing the viral antigen and is enzyme catalyzed, transforming into a colorless chromogen pigmented compound. For this purpose, ID Screen® Akabane Competition ELISA (ID Vet, Product Code: AKAC, Lot No: F42, France) commercial kit produced by ID Vet company was used. The test was performed according to the kit procedure reported by the manufacturer (ID Vet). The results were evaluated in an ELISA reader (Mindray MR-96A, Hamburg-Germany) using a 450 nm filter. These absorbance values obtained were calculated as specified in the kit's protocol.

In the evaluation of the samples; The result was calculated by multiplying the ratio of the optical density (OD_{Sample}) value of the plate eye on which the sample was placed to the negative control optical density (OD_{NC}) value by 100, and the result

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was evaluated according to the table specified in the test procedure. This process was calculated for each sample separately and the status of each sample was determined (positive/negative) according to the values specified in the table (S/N % = (OD_{Sample} ÷ OD_{NC}) x 100). Using the table below according to the test procedure, each blood serum sample was evaluated. The sample was considered negative if the result was greater than or equal to 30%. The sample was considered positive if it was less than 30% (Table 2).

Table 2. AKAV C-ELISA test results and evaluation.

Result	Evaluation
S/N < 30%	Positive
S/N ≥ 30%	Negative

Statistical Analysis

Statistical analysis was carried out with Statistical Package for Social Sciences software (IBM SPSS 21 Software, USA). Chi-square (chi-square -χ2) test was used to evaluate the statistical significance of the difference between the seropositive rates detected in the sampling districts, the difference between the seropositive rates determined in males and females, and the difference between the AKAV seropositive rates determined in age groups. A p-value < *0.*05 was regarded as significant difference.

Results

The seroprevalence rate of AKAV infection was determined as 2.12% (9/425) in the blood serum of 425 pure Honamli goats tested in the study. When the antibody positivity rates were evaluated on the basis of districts, the highest seropositivity was found in Karamanlı with a rate of 6.67% (5/75). Among its other districts, 4% (2/50) seropositivity was found in Bucak, 1.82% (1/55) in Çeltikçi, and 1.79% (1/56) in Ağlasun (Figure 2). No positivity was detected in other centers where sampling was performed.

The distribution of 9 seropositive samples by gender was found to be 1.37% (1/73) in males and 2.27% (8/352) in females (Table 3).

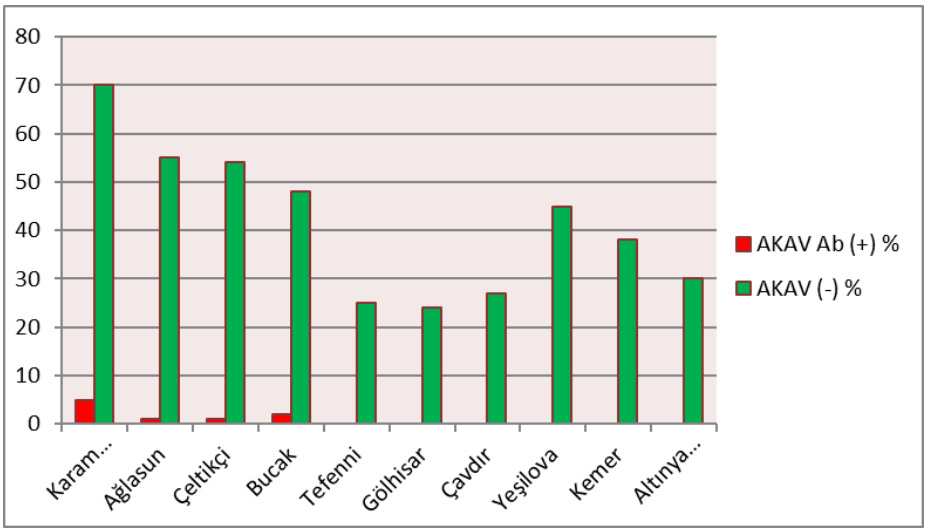

Figure 2. Districts and seropositivity rates in Burdur province

AKAV seropositivity rates were determined according to the age of Honamli goats sampled in the study. Accordingly, 3.66% (3/82) in 4 year old goats, 3.70% (3/81) in 5 year old goats, 3.45% (2/58) in 6 year old goats and 3% in 7 year old goats. Antibody positivity was detected at a rate of 3.33 (1/30). Seropositivity was not determined in one, two, and three-year-old animals (Table 4.)

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In the evaluation of the barn condition in which the goat herds belonging to the sampled private small scale family production units are housed, wall/roof: present, absent criteria; floor quality: with low, moderate and good grades; drinker status: low, moderate and good; insect control: done, not done criteria; Separate housing compartment for goats, kids and goats: evaluated with the criteria of present, absent, and the determined results are shown in Table 5. In addition, it was determined that there were occasional abortion cases in all of the sampled goat herds and the natural breeding method was used in insemination.

Statistical Analysis Results

As a result of the statistical analysis, it was determined that the difference between the AKAV seropositivity rates in the districts of Burdur, where the samples were collected, was statistically insignificant *(p>0.05)* ($\chi2$=2.894, p=0.408). Again, it was determined that the difference in positivity rates in males and females and the differences in positivity rates in different age groups were statistically insignificant *(p>0.05)* (Male/female $\chi2$=0.238, p=0.626; age groups $\chi2$=0.013, p=1,000).

Discussion

Various of diseases threatening human and animal health (Akabane, Crimean Congo fever, West Nile virus, Sandfly fever) are usually transmitted by arthropods feeding on the blood such as mosquites, ticks and sandflies. Due to rapid development of modern transport systems, global warming, demographic changes, the disappearance of natural ecological borders because of fast urbanization and the emergence of new areas increasing contact between vector species and their hosts, very important health problems start to occur in new millennium such as arboviruses.

Table 3. AKAV seroprevalence rates by gender

Gender	Number of samples (n)	AKAV Ab		AKAV Ab	
		n (+)	%	n (-)	%
Male	73	1	1.37	72	98.63
Female	352	8	2.27	344	97.73
Total	**425**	**9**	**2.12**	**416**	**97.88**

Table 4. AKAV seropositivity rates by age groups

Age	Number of samples (n)	AKAV Ab			
		n (+)	%	n (-)	%
6-12 month	38	0	0	38	100
2 age	62	0	0	62	100
3 age	74	0	0	74	100
4 age	82	3	3.66	79	96.34
5 age	81	3	3.70	78	96.30
6 age	58	2	3.45	56	96.55
7 age	30	1	3.33	29	96.67
Total	**425**	**9**	**2.12**	**416**	**97.88**

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Table 5. Evaluation of the Conditions of the Barns Housing the Sampled Honamli Goat Herds

Sampling Districts	Wall-roof availability	Floor quality	Drinker status	Insect control	Separate compartment (for goats, kids and goats)
Karamanlı/Burdur	Absent	Low	Low	No	Absent
Ağlasun/Burdur	Present	Moderate	Low	No	Absent
Çeltikçi/Burdur	Absent	Low	Low	No	Absent
Bucak/Burdur	Absent	Low	Low	No	Absent
Tefenni/Burdur	Present	Good	Good	No	Present
Gölhisar/Burdur	Present	Good	Good	No	Present
Çavdır/Burdur	Present	Good	Good	No	Present
Yeşilova/Burdur	Present	Good	Good	No	Present
Altınyayla/Burdur	Absent	Moderate	Moderate	No	Present
Kemer/Burdur	Present	High	High	No	Present

When viewed from this aspect, geographical localization of the research area is extremely important. Burdur province, where the study was conducted, is in West of Mediterranean Region of Turkey, located at 37°43' North 30°17' East coordinates and its altitude is 950 meters. There are rich water sources, rivers and lakes in the province, hence the area is called the region of lakes. Next to the city of Burdur, "Burdur Lake" is located and is the seventh largest lake of Turkey with coordinates 37°45' North, 30°12' East and an area of 250 km² (2013 DSİ). Moreover, there are various kinds of natural lakes and dam lakes established for irrigation (Karatas Lake, Karamanli Lake, Onac Lake, etc…) in the region. Likewise, irrigation-based agriculture is actively applied in the region as well as animal husbandry. Considering all these geographical aspects, it is safe to say Burdur province is ideal environment for vector mosquitoes' habitats due to the epidemiological cycle of AKAV.

The climatic features of the region are also suitable for AKAV. The annual average temperature is 13.2°C and the annual average number of rainy days is 89 (Hızel et al., 2010). It is thought that the changes in the annual average temperature and the annual average number of rainy days are the result of the altitude and topographic structure. The area, where the study conducted, provides a suitable habitat for mosquito larvae with its wide variety of wetlands and contains different type of hosts that adults can feed on due to intensive livestock activities. All this features makes the region potential risky area for Akabane virus infection.

Furthermore, Antalya province, the capital of tourism, next to Burdur province, has suitable habitats for AKAV vectors. Due to the fact that Antalya International Airport, marina, highway connections of the coastline passing through this region and the climate of the region provides an appropriate enviroment, direct transfer of virus-infected vector mosquitoes from other regions/countries to these regions poses a potential risk. Thus, there is a possibility of transmission of Akabane virus from close provinces to Burdur province.

There are many studies on Akabane infection in Turkey and in the world. In one study, AKAV seropositivity was determined as 87% (47/54) in the blood serum of 54 cattle with AH syndrome in Israel. Same study also reported that AKAV seropositivity in farms without AH syndrome was 3.7% (1/27). According to the aforementioned study, AKAV vector mosquites were active between August and December, thus spreading AKAV (Brenner et al., 2004). General prevalence

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rates of AKAV antibodies in dairy cattles in Sudan was reported as 29.4% and prevalence rate was between 69.6% and 3.3%among the states. Similarly, the prevalence of AKAV antibodies was higher in crossbred animals than in domestic animals, and a higher rate of positivity was detected in females than males. At the same time, the prevalence of AKAV has been reported to be high in infertility and abortion cases (Elhassan et al., 2014). In another study, 210 blood serum samples collected from affected cattle during the cattle enzootic encephalomyelitis epidemic in 2010 were analysed for AKAV antibody by using serum neutralization test (SNT) and ELISA. As a result, seropositivity rates of SNT and ELISA were determined as 90.0% and 85.2%, respectively (Oem et al., 2014).

The very first report of Akabane disease in Turkey was delivered by Urman et al. in 1980. In the following years, many studies have been carried out to evaluate the status and prevalence of Akabane infection. In two different studies, AKAV seroprevalence rate in cattles was determined as 13.7% (Çabalar and Dağalp, 2006) and 27.98% (Özgünlük, 2003) in Southeastern Anatolia region. Moreover, Akabane seropositivity was reported at a rate of 22% in the Black Sea region (Albayrak and Özan, 2010) and 0.14% in the Thrace region (Karaoğlu et al., 2007). Although AKAV seropositivity of cattle was reported as 9.72% in Aydın province in the Aegean region (Özgünlük et al., 2013), seropositivity was not found in sheep, goats and cattle in another study conducted in the same province (Koç, 2014). Similarly, meanwhile in a study related to Akabane infection in small ruminants (sheep and goats) seropositivity was reported as 1.1% in goats in Aydın province of Aegean region, seropositivity could not be detected in Muğla (Tan and Bilge, 2000). The seropositivity of Akabane infection was reported as 0.08% in sheep in the Marmara region (Pestil, 2014) and 44.9% in sheep in the Mediterranean region (Şevik, 2017). In a seroprevalence study conducted in Hatay, seropositivity rates were found as 42.41% in cattle, 16.19% in sheep, and 7.46% in goat (Doğan, 2018).

In this study, seroprevalence of AKAV infection was determined as 2.12 % (9/425). In the districts where the research was conducted, these seropositivity rates were 6.67% in Karamanlı, 1.79% in Ağlasun, 1.82% in Çeltikçi, and 4.00% in Bucak seperately. In addition, the distribution of seropositivity in our study by gender was 1.37% in males and 2.27% in females.

In husbandries with seropositivity, it was identified that wall, roof and floor condition of barns where animals live in, are uneven, sloppy and dirty; feeders and water sources are not hygienic; there is no pest control administration, there is no separate section for offsprings and all animals are living together. The seropositivity rate of Honamli goat breed in our study is coherent with previous studies conducted on small ruminants, thus results are significant and valuable. The Mediterranean region has a suitable geographical location and climatic conditions for the survival of *Clucoides species*, which are an important vector in the transmission and transmission of the agent.

Depending on breeding purpose of goats (meat, milk or mixed), the status of AKAV infection may vary. It has been determined that the transmission of Akabane virus from mother to offspring during pregnancy is more common in beef breed goats. Likewise, AKAV infection transmission by mosquitoes and spreading among animals are more likely in dairy goats raised in crowded, closed and cramped enviroments and not left outside for grazing. Thus barn conditions, husbandry practices and indoor goat breeding have an important role in spreading the infection.

The increase of greenhouse gas density in the atmosphere in the last century results in the melting of glaciers and droughts associated with the global warming. Thus these effects of the global warming cause changes in habitats of animals and forces them either to adapt the current situations or to live in higher regions. Due to all these reasons, the potential of mosquitoes to spread the disease, which is the vector of Akabane virus as a result of atmospheric changes, is increasing day by day.

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Consequently, considering the data of this study and presence/prevalence of Akabane infection in the region where the study was conducted, it is possible to report that the disease is important both in Burdur and other regions of Turkey in terms of animal husbandry, and serious measures should be taken to prevent infection. The most important factor in preventing the spread of the disease is the struggle against vectors. Traps to be set in areas where biting midges are common will both prevent the spread of infection and enable more detailed research to be carried out by identifying the species of the caught flies. In addition, it has been concluded that the vaccination studies against AKAV, which is applied in some countries where the disease is seen but not yet implemented in our country, will be useful to define the places where the infection is seen in our country.

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