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Seroepidemiological Investigation of Maedi-Visna Virus Infection in Kangal Akkaraman Sheep Raised in the Sivas Region

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ABSTRACT

Maedi-visna virus (MVV) is a retrovirus belonging to the Lentivirus genus that causes chronic and progressive disease in sheep with high global prevalence. This infection affects the respiratory and nervous systems of animals, leading to chronic pulmonary complications and neurological disorders. Currently, no therapeutic interventions or prophylactic vaccines are available for MVV infection. The objective of this study was to determine the seroprevalence of MVV infection in Kangal Akkaraman breed sheep raised in the Sivas region. Within the scope of the research, a total of 460 blood samples were collected using random sampling methodology, selecting five farms from each district across Sivas city center and ten districts. Commercial ELISA assay was employed for the detection of MVV antibodies. Serological analysis using ELISA revealed seropositivity in 228 out of 460 examined animals (49.6%). District-based seroprevalence rates demonstrated considerable variation, ranging from 13.04% (Gürün) to 82.60% (Gemerek). Age group analysis revealed a progressive increase in seropositivity rates with advancing age. Seropositivity was determined as 30% in male animals and 50% in female animals. Statistical evaluation indicated significant differences between districts and age groups ($p<0.001$ and $p<0.05$, respectively), while differences between farms and sexes did not demonstrate statistical significance ($p>0.05$). The obtained data indicate that Maedi-visna infection exhibits high prevalence in the Sivas region and may lead to substantial economic losses. This situation emphasizes the necessity for detailed investigation of the epidemiological characteristics of the disease, expansion of prevalence studies, and support of viral genotyping analyses through comprehensive field studies.

Keywords: Epidemiology, Kangal Akkaraman, Maedi-Visna Virus, Sivas

Sivas Yöresinde Yetiştirilen Kangal Akkaraman Koyunlarında Maedi Visna Virüs Enfeksiyonunun Seroepidemiyolojik Olarak Araştırılması

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ÖZ

Maedi-visna virüsü (MVV), dünya genelinde yüksek prevalansa sahip, koyunlarda kronik ve progresif hastalığa neden olan Lentivirus cinsine ait bir retrovirüstür. Bu enfeksiyon, hayvanların solunum ve sinir sistemlerini etkileyerek kronik akciğer problemleri ve nörolojik bozukluklara yol açar. MVV enfeksiyonunun tedavisi ve aşısı bulunmamaktadır. Bu çalışmada Sivas bölgesinde yetiştirilen Kangal Akkaraman ırkı koyunlarda MVV enfeksiyonunun seroprevalansının belirlenmesi hedeflenmiştir. Araştırma kapsamında Sivas merkez ve on ilçesindeki işletmelerden rastgele örnekleme yöntemiyle her ilçeden beş işletme seçilerek toplam 460 kan örneği toplanmıştır. MVV antikorlarının tespiti için ticari ELISA testi kullanılmıştır. ELISA testi kullanılarak incelenen 460 hayvanın 228'inde (%49,6) seropozitiflik tespit edilmiştir. İlçeler bazında seroprevalans oranları %13,04 (Gürün) ile %82,60 (Gemerek) arasında değişkenlik göstermiştir. Yaş grupları analizi, seropozitiflik oranının yaşla birlikte artış sergilediğini ortaya koymuştur. Erkek hayvanlarda %30, dişi hayvanlarda %50 seropozitiflik belirlenmiştir. İstatistiksel değerlendirmede ilçeler arası ve yaş grupları arası farklılıklar anlamlı bulunurken ($p<0,001$ ve $p<0,05$), işletmeler arası ve cinsiyetler arası farklılıklar istatistiksel anlamlılık göstermemiştir ($p>0,05$). Elde edilen veriler, Maedi-visna enfeksiyonunun Sivas bölgesinde yüksek prevalans gösterdiğini ve önemli ekonomik kayıplara yol açabileceğini işaret etmektedir. Bu durum, hastalığın epidemiyolojik karakteristiklerinin detaylı araştırılması, prevalans çalışmalarının genişletilmesi ve viral genotiplendirme analizlerinin kapsamlı saha çalışmaları ile desteklenmesi gerekliliğini ortaya koymaktadır.

Anahtar Kelimeler: Epidemiyoloji, Kangal Akkaraman, Maedi Visna Virüs, Sivas

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Giriş

The domestication of sheep began in Mesopotamia around 8000 BC and spread to other continents during the Neolithic period (Çıvgın, 2024; Zeder, 2008). Türkiye's geographical conditions and climatic characteristics are highly favorable for small ruminant livestock farming, which requires minimal feed and possesses the capability to produce meat, milk, leather, and wool (Bilginturan, 2009). Sheep farming in Türkiye represents an economically significant sector with 42,060,470 head of sheep, 489,354 tons of meat production, and 1,067,342 tons of milk production (TUİK, 2024). The Akkaraman sheep breed is extensively raised in the Central Anatolia Region and demonstrates high adaptation to regional conditions (Akçapınar, 2000). The Kangal Akkaraman breed emerged through natural selection and was recognized as a distinct breed in 2012 (Oğrak, 2020).

Maedi-Visna Virus (MVV) is a lentiviral agent that affects the respiratory and nervous systems in sheep and is characterized by a prolonged incubation period (Palsson, 1976; Sigurdsson, 1954). MVV, being the first isolated lentivirus, belongs to the Lentivirus genus of the Orthoretrovirinae subfamily within the Retroviridae family and has been renamed Lentivirus ovivismae by the International Committee on Taxonomy of Viruses (ICTV) (ICTV, 2024). Initially, it was believed that MVV infected only sheep while Caprine Arthritis Encephalitis Virus (CAEV) infected only goats. However, recent molecular epidemiological studies have demonstrated that these viruses represent variants that infect both sheep and goats. Due to the possibility of interspecies infection and recombination, this viral cluster is now designated as small ruminant lentiviruses (SRLV) (Blacklaws, 2012; Thormar, 2013).

Lentiviruses are retroviruses that are widespread globally and cause chronic and fatal diseases with prolonged incubation periods in humans and other mammalian species (Larruskain & Jugo, 2013). SRLVs have been classified into five genotypes (A, B, C, D, E) using gag, gag-pol genes and LTR sequences (Michiels et al., 2020). Genotypes A and B are MVV-like and CAEV-like strains that predominate worldwide; genotypes C, D, and E have been isolated only in Europe (Reina et al., 2010; Glaria et al., 2012). Recent phylogenetic studies suggest that SRLV-genotype A may have emerged in a region within Türkiye's borders and spread worldwide through human migration movements (Muz et al., 2013; Carrozza et al., 2018).

Numerous serological studies on Maedi-Visna virus (MVV) infections have been conducted across different regions and various sheep breeds in Turkey, predominantly employing ELISA testing, with these investigations demonstrating seropositivity rates ranging from 0% to 80%. (Çelik et al., 2018; Ün et al., 2018; İnce, 2020; Kuruçay et al., 2024). This study was conducted to determine the seroprevalence of Maedi-Visna infection in Kangal Akkaraman sheep, an important sheep breed of Turkey that is extensively raised in Sivas province. This study is the first comprehensive study conducted on Kangal Akkaraman sheep breed and will contribute to the development of strategies for the management and control of the disease by determining the prevalence of infection in the population.

Material and Method

The study was conducted following approval from the Local Ethics Committee for Animal Experiments of Sivas Cumhuriyet University (Decision No: 65202830-050.04.04-63 Date: 29.03.2024) and the Provincial Directorate of Agriculture and Forestry (Approval No: E-80110403-325.04.02-13457279).

In this study, to determine the MVV seroprevalence in Kangal-Akkaraman breed sheep in the Sivas region, ten districts where this breed is intensively raised and animal movement is frequent were selected, and blood samples were collected from a total of 460 sheep from five different farms in each district (Table 1). The sampling strategy primarily targeted breeding female animals aged two years and older, while breeding rams and ram candidates used for mating were also included through random sampling. Animals selected for the study were clinically healthy individuals that had not received medication against any infection in the recent period. Blood samples were collected from the Vena jugularis under aseptic conditions using sterile vacuum tubes and transported to the laboratory under cold chain conditions. Blood samples were centrifuged at 3000 rpm for 10 minutes to separate sera, which were then transferred to Eppendorf tubes and coded according to the region of origin and ear tag numbers. Serum samples were subjected to inactivation at 56°C for 30 minutes and stored at -20°C until testing. The test was performed using a commercial ELISA test kit (ID Screen®, MVV/CAEV Indirect, ID-Vet, France) according to the manufacturer's instructions, and results were evaluated using an ELISA reader at 450 nm wavelength.

Table 1. Districts and Number of Farms and Animals Sampled

District	Number of Farms	Number of Animals
Şarkışla	5	46
Gemerek	5	46
Gürün	5	46
Kangal	5	46
Altınyayla	5	46
Hafik	5	46
Zara	5	46
Ulaş	5	46
Merkez	5	46
Yıldızeli	5	46
TOTAL	**50**	**460**

All statistical analyses were performed using R programming language version 4.5.0 (R Core Team 2025). Chi-square (X^2) test was applied to evaluate the relationship between categorical variables. In cases where expected cell frequencies were below 5, Fisher's exact test was employed for analysis. P values <0.05 were considered statistically significant.

Results and Discussion

As a result of our study conducted in the Sivas region, it was determined that MVV disease, which follows a subclinical course in Kangal Akkaraman breed sheep, is widespread with a seroprevalence of 49.56% (228/460) (Table 2).

Table 2. MVV Seroprevalence Rates by District

District	Positive	Negative	Seropositivity (%)	P value
Şarkışla	21	25	45.65	
Gemerek	38	8	82.60	
Gürün	6	40	13.04	
Kangal	9	37	19.56	
Altınyayla	22	24	47.82	
Hafik	17	29	36.95	<0.001
Zara	36	10	78.26	
Ulaş	25	21	54.34	
Merkez	36	10	78.26	
Yıldızeli	18	28	39.13	
TOTAL	**228**	**232**	**49.56**	

According to the test results of samples collected from 10 different districts, these values were determined as 45.65% in Şarkışla, 82.60% in Gemerek, 13.04% in Gürün, 19.56% in Kangal, 47.82% in Altınyayla, 36.95% in Hafik, 78.26% in Zara, 54.34% in Ulaş, 78.26% in the city center, and 39.13% in Yıldızeli (Table 2). The differences in positivity rates between districts were found to be statistically significant (p<0.001).

When the results were examined on a farm basis, positivity was detected in 40 out of 50 farms (80%) selected from 10 districts. While positivity was detected in all five farms (100%) selected from Gemerek, Zara, Ulaş, and the city center, the lowest positivity rate was detected in Kangal with two out of five farms (2/5) (40%). Positivity was detected in three out of five farms (60%) in Şarkışla and Hafik districts, and in four out of five farms (80%) in Gürün, Altınyayla, and Yıldızeli districts (Table 3). The differences in positivity rates between farms were not found to be statistically significant (p>0.05).

When MVV seroprevalence rates were classified according to age groups, they were determined as 16.6% (1/7) in one-year-old animals, 33.3% in two-year-olds, 40.54% (30/74) in three-year-olds, 44.34% (51/115) in four-year-olds, and 56.35% (142/252) in animals aged five years and above (Table 4). Upon evaluation of the results, an increasing prevalence rate with age is observed. The difference between positivity rates according to age was found to be statistically significant (p<0.05).

Table 3. MVV Seroprevalence Rates by Farm

District	Positive Farms/Total Number of Farms	Positivity Rates (%)	P Value
Şarkışla	3/5	60	
Gemerek	5/5	100	
Gürün	4/5	80	
Kangal	2/5	40	
Altınyayla	4/5	80	
Hafik	3/5	60	0.261
Zara	5/5	100	
Ulaş	5/5	100	
Merkez	5/5	100	
Yıldızeli	4/5	80	
TOTAL	**40/50**	**80**	

Table 4: MVV Seroprevalence Rates by Age

Age	Positive	Negative	Total	Prevelance (%)	P Value
1	1	6	7	16.6	
2	4	8	12	33.3	
3	30	44	74	40.54	
4	51	64	115	44.34	0.011
5 and above	142	110	252	56.35	
Total	**228**	**232**	**460**	**49.56**	

When examined by gender, MVV seroprevalence was determined as 30% (3/10) in male animals raised as rams or ram candidates in the farms, and 50% (225/450) in females (Table 5). The difference between positivity rates according to gender was found to be statistically non-significant (p>0.05).

Table 5: MVV Seroprevalence Rates by Gender

Gender	Positive	Negative	Positive (%)	P Value
Male	3	7	30	
Female	225	225	50	0.339
Total	**228**	**232**	**49.56**	

MVV infection emerged as an epidemic showing progressive pneumonia and paralysis symptoms in Iceland in the 1930s, but the cause of the disease was not understood until the 1950s. In this epidemic, approximately 150,000 sheep died from the infection, and 650,000 sheep were culled for disease control purposes (Sigurdsson, 1954). Although the first virus isolation was performed in Iceland, studies related to compatible lesions were published in the Netherlands (Houwers, 1990). In North America, CAEV showing antigenic similarity to MVV was isolated in 1980 (Crawford et al., 1980). The global spread of the disease occurred through importation (Pritchard et al., 1984). Phylogenetic analysis of SRLVs indicates that the pandemic spread of genotypes A and B occurred recently due to international livestock trade (Shah et al., 2004). The virus spread to European countries between 1960-1990, and its presence has been proven in all countries except Iceland, New Zealand, and Australia today (Kalogianni et al., 2020).

In a comprehensive study conducted in Spain in 2010, 274,048 sheep were examined, 52.83% seropositivity was determined, and a control plan based on seronegative breeding stock selection and gradual culling of seropositive animals was implemented (Pérez et al., 2010). In a meta-analysis study published in 2021, it was reported that SRLV infection is widespread worldwide, with the highest individual prevalence (40.9%) particularly in Europe (De Miguel et al., 2021).

MVV infection was first detected in Türkiye in 1975 in slaughterhouse studies (Alibaşoğlu & Arda, 1975). In 1985, the disease was definitively identified in rams imported from Germany (Girgin et al., 1987). All studies conducted in Türkiye until 2011 were studies that serologically demonstrated the presence of MVV infection. While most of the studies conducted especially until 2002 were performed using the AGID test, studies conducted after 2002 were performed using the ELISA test.

In the first large-scale study conducted by Burgu (1990), 23.9% seropositivity was detected in 1099 serum samples, with positivity found in 10 out of 12 farms. Flock-based positivity rates ranged between 1.5-56.2% (Burgu, 1990). While Alkan & Tan (1998) reported rates ranging between 3.8-41.2% in six flocks, Tan & Alkan (2002) detected 26.7% individual positivity in 628 samples, with positivity found in 9 out of 10 farms. In studies where lower rates were reported, Karaoğlu et al. (2003) detected 2.6% in 825 sheep, and Yavru et al. (2012) detected 2.9% individual positivity. While no infection was encountered in some regions (Kandil et al., 1997; Aslantaş et al., 2002), studies using AGID tests generally reported 0-26.7% individual and 0-90% flock-based positivity.

In the first ELISA study conducted in Türkiye, Schreuder et al. (1988) found a positivity rate of 1.5% in Erzurum. A significant increase in seropositivity rates was observed in subsequent years. Çimtay et al. (2004) reported a positivity rate of 10% in Şanlıurfa, and Preziuso et al. (2010) reported a positivity rate of 15.3% in Istanbul. Albayrak et al. (2012) found a relatively high rate of 69% in Herik sheep in the Black Sea Region. Among studies reporting moderate positivity, Arslan et al. (2012) found a positivity rate of 19.7% in Şanlıurfa, Azkur et al. (2011) found a positivity rate of 19.4% in Kırıkkale and Gürçay and Parmaksız (2013) found a positivity rate of 9.8% in Şanlıurfa. In recent studies, Ameen and Karapınar (2018) reported a positivity rate of 10.5% in Van, Gezer et al. (2021) 16% in Kars, Kalaycı et al. (2023) 24.42% in the Aegean Region, Timurkan et al. (2024) 15,12% in Erzurum and Kuruçay et al. (2024) 55,25% in Central Black Sea Region.

When compared with studies conducted in Turkey, the seropositivity rates show similarity to those in two studies conducted particularly in the Black Sea region (Albayrak et al., 2012; Kuruçay et al., 2024) and indicate quite high positivity. This situation may be attributed to the geographical proximity of Sivas to the studied region. However, when compared with findings obtained in studies conducted in other regions recently (Ameen and Karapınar, 2018; Gezer et al., 2021; Kalaycı et al., 2023; Timurkan et al., 2024), the considerably lower seropositivity rates in these regions compared to the results obtained in our study strengthens this hypothesis.

The first molecular diagnosis of MVV in Türkiye was performed by Akkoc et al. (2011) using PCR in a young sheep that died with neurological symptoms. Muz et al. (2013) reported 51% seropositivity in 911 blood samples and characterized the LTR and gag gene regions of Turkish MVV isolates by PCR. Doğan et al. (2021) detected 9.43% positivity in 700 samples and confirmed the presence of genotype B, while Sait & İnce (2022) demonstrated that A-type lentivirus was in circulation in Yalova.

In our study, 49.56% MVV positivity was detected in 460 Kangal-Akkaraman breed sheep in Sivas province. This rate indicates a moderate-to-high prevalence compared to previous studies in Türkiye. According to districts, the highest rates were detected in Gemerek (82.60%), Merkez (78.26%), and Zara (78.26%), while the lowest rate was detected in Gürün (13.04%). In the evaluation according to age groups, the rate of 16.6% in one-year-old animals reached 56.35% in animals aged five years and above, indicating that the infection increases with age. This situation is consistent with the literature (Preziuso et al., 2010; Gezer et al., 2021; Kalaycı et al., 2023). In the evaluation according to gender, 50% seropositivity was detected in female animals and 30% in male animals. The high rate in females can be explained by the longer use of these animals in breeding and their greater contact with infected animals. This finding is also consistent with the literature (Preziuso et al., 2010).

The MVV seropositivity of 49.56% detected in Kangal-Akkaraman breed sheep in Sivas province in this study indicates that the infection is widespread at a serious level in the region. Inter-district prevalence differences (13.04-82.60%) reveal that flock management and geographical factors are effective in the spread of the disease. Seropositivity rates increasing with age (from 16.6% to 56.35%) and high prevalence in female animals (50%) confirm the cumulative character of the infection. The presence of MVV infection in Türkiye has been known since 1975, and studies have reported seroprevalence rates ranging from 0-80%. Molecular studies have shown that both A and B genotypes are in circulation in our country and revealed the phylogenetic relationship of Turkish isolates with global strains (Muz et al., 2013; Doğan et al., 2021; Sait & İnce, 2022).

Suggestions and Conclusion

Systematic serological and molecular screening programs covering the entire country should be implemented to determine the true extent of MVV infection in Türkiye. This program will reveal regional prevalence differences in detail and contribute to understanding the epidemiological characteristics of the disease. Characterization of the genetic features of MVV strains detected in Türkiye in larger sample groups will provide critical information about the evolution, transmission dynamics, and regional adaptations of the disease. Particularly, phylogenetic data obtained through sequence analysis of the gag gene will enable comparative analyses with global strains. As in the Spanish example (Pérez et al., 2010), a national program should be developed that includes seronegative breeding animal selection, gradual removal of seropositive animals from flocks, and flock-based control measures. This program will enable the elimination of MVV from infected flocks without the need for culling entire herds. Since MVV-infected animals generally remain asymptomatic, producers may be unaware of the disease's presence. Therefore, comprehensive education programs for farmers should be organized to raise awareness about transmission routes, protection methods, and economic impacts of the disease. To prevent flock-to-flock transmission, serological testing requirements should be implemented for animal trading, quarantine practices should be standardized and effectively monitored. These measures should be applied more strictly, particularly in breeding animal trade. Laboratory infrastructure should be strengthened to increase both serological and molecular diagnostic capacity, and the widespread use of PCR-based diagnostic methods should be ensured. Early diagnosis capability is of critical importance in controlling the disease. Regular monitoring studies should be conducted to evaluate the effectiveness of implemented control programs, and systematic analysis of obtained data should be ensured.

In conclusion, MVV infection is a disease that is widespread at a level that cannot be ignored and economically harmful for Turkish livestock. The high seroprevalence rates detected in Kangal Akkaraman breed sheep raised in Sivas province in our study indicate that the disease constitutes a serious problem at the regional level. To control the infection, long-term and sustainable control strategies integrating serological and molecular diagnostic methods must be implemented. The national control and eradication program to be developed will not only reduce the spread of MVV infection but also make significant contributions to the sustainability, productivity, and international competitiveness of the livestock sector. In this context, cooperation between public institutions, universities, and sector representatives is of critical importance for achieving successful results.

Conflict of Interest Statement

The authors have no relevant financial or non-financial interests to disclose.

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