



## RESEARCH ARTICLE

### Investigation of seroprevalence of maedi-visna disease in sheep flocks in Afyonkarahisar province

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### Afyonkarahisar ili koyun sürülerinde maedi-visna hastalığı seroprevalansının araştırılması

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#### Öz

**Amaç:** Bu çalışmada, Afyonkarahisar ilindeki koyunlarda Maedi-Visna Virus (MVV) enfeksiyonunun varlığı serolojik olarak belirlenerek, söz konusu bölgede MVV enfeksiyonu ile ilişkili risk faktörlerinin analiz edilmesi amaçlandı.

**Gereç ve Yöntem:** Bu amaçla bölgeden epidemiyolojik örneklem büyüklüğü göz önünde bulundurularak, 22 koyun sürüsünden 248 adet serum örneği Mayıs 2016- Nisan 2018 yılları arasında rastgele toplandı. Toplanan örnekler, MVV spesifik antikorları için ELISA kitiyle test edildi. Potansiyel MVV risk faktörleri istatistiki olarak tek değişkenli logistik regresyon analizi ile değerlendirildi.

**Bulgular:** Örneklenen hayvanlarda seropozitivite değeri %5.65 (14/248), sürü bazında %13.63 (3/22) olarak bulundu. Seropozitiflik değeri dişi ve erkeklerde sırasıyla %3.94ve 13.33 olarak belirlendi. Hayvan bazında, sürüdeki keçi varlığı ve cinsiyet ile hastalık enfeksiyonunun seropozitifliği arasında istatistiki açıdan anlamlı bir ilişki tespit edildi ( $p < 0.05$ ).

**Öneri:** Koyunculuk sektöründe ekonomik kayıplara yol açan, koyunların yaş seyrirli, giderek şiddeti artan özellikle, persiste ve ölümle son bulan bir viral hastalığı olan MV enfeksiyonu tespit edilen sürüler, ekonomik kayıplara yol açan viral etkenler yönünden periyodik olarak takip edilmelidir. Bu çalışmanın sonuçları göz önünde tutularak, MV enfeksiyonunun epidemiyolojisinin sürülerde düzenli izlenerek analiz edilmesi ve kontrolüne ilişkin stratejilerin geliştirilmesinin faydalı olacağı düşünülmektedir.

**Anahtar kelimeler:** ELISA, koyun, Maedi-Visna, risk faktörü, seroprevalans

#### Abstract

**Aim:** In this study, it was aimed to determine the presence of Maedi-Visna Virus (MVV) infection in sheep in Afyonkarahisar province serologically and to analyze the risk factors associated with MVV infection in the region.

**Materials and Methods:** For this purpose, 248 serum samples were randomly collected from 22 sheep flocks considering the epidemiological sample size from the region between May 2016 and April 2018. Collected samples were tested with the ELISA kit for MVV specific antibodies. Potential MVV risk factors were evaluated statistically by univariate logistic regression analysis.

**Results:** The seropositivity in the ensampled animals was found to be 5.65% (14/248), and it was 13.63% on the basis of flock (3/22). Seropositivity was determined to be 3.94% and 13.33% in males and females, respectively. On the basis of animals, there was a statistically significant relationship between the presence of goat in the flock and gender and seropositivity of the disease infection ( $p < 0.05$ ).

**Conclusion:** Flocks in which sheep with persistent MV infection with a viral disease which is characterized by slow progress followed by a gradual increase in severity and results in death leading to economic losses in the sheep sector are detected, should be monitored periodically for viral factors leading to economic losses. Considering the results of this study, it is thought that analyzing epidemiology of MV infection in flocks through regular monitoring and developing strategies for controlling it would be useful.

**Keywords:** ELISA, Maedi-Visna, risk factors, seroprevalence, sheep



## Introduction

Maedi-Visna (MV) is an infection occurred by the Maedi-Visna Virus (MVV) containing a single-stranded RNA (+) classified in the genus *Lentivirus* in the Retroviridae family, which is characterized by the respiratory tract and affects especially sheep. It is observed in two different forms as respiratory and nervous system forms (Gomez et al 2018, Scott 2019). MVV and Caprine arthritis-encephalitis virus (CAEV) have a very close antigenic affinity and are called Small Ruminant Lentiviruses (SRLV) (Santry et al 2013). Caprine arthritis and encephalitis (CAE) is the name of the disease observed in goats, in which the joints and the nervous system are most frequently affected (Leroux et al 2013). MVV was first detected in Iceland in 1939. It causes a slowly progressing pneumonia localized in lungs in the maedi form, while the central nervous system is affected by causing paralysis in the visna form (Gomez et al 2018, Scott 2019).

The modes of transmission in the epidemiology of infection are of great importance. Intake of infected colostrum or milk and droplet infection are of great importance in the transmission of MV infection (Blacklaws 2012). The agent passes into colostrum and milk through infected macrophages in the mammary gland. In particular, lambs receive the infection in this way (Blacklaws 2012, Gomez et al 2018).

For serological diagnosis, complement fixation (CF), indirect immunofluorescence (IIF), agar gel immunodiffusion (AGID), indirect ELISA, Western blot and radio-immunoprecipitation tests are used (De Andres et al 2013, OIE 2017). MV is a slowly progressing infection, and despite the humoral and cellular response to the virus, it is difficult to control and eradicate the disease, as the virus remains persistent throughout its lifetime (Peterhans et al 2004, Oguma et al 2014). MV infection is observed in all countries except Australia and New Zealand (OIE 2017, Gomez et al 2018). The presence of MVV infection in Turkey dates back to the 1975s (Alibaşoğlu and Arda 1975). In Turkey, its seroprevalence was reported to be between 2.9% and 28.1% in studies based on MVV specific antibody detection (Gürçay and Parmaksız 2013, Un et al 2018). MV infection is an economically important disease because it causes a live weight loss, and a decrease in fertility and milk yield in sheep breeding (Arsenault et al 2003, Perez et al 2010, White and Knowles 2013).

In this epidemiological study, it was aimed to evaluate the epidemiology of infection in a broader framework and to make recommendations for the fight against infection by sampling from enterprises in the Afyonkarahisar Province in Turkey and analyzing the risk factors related to the disease.

## Material and Methods

### Study area and design

This study was carried out in the Afyonkarahisar province between latitude 38.39.06 and longitude 30.40.12 (Figure 1).

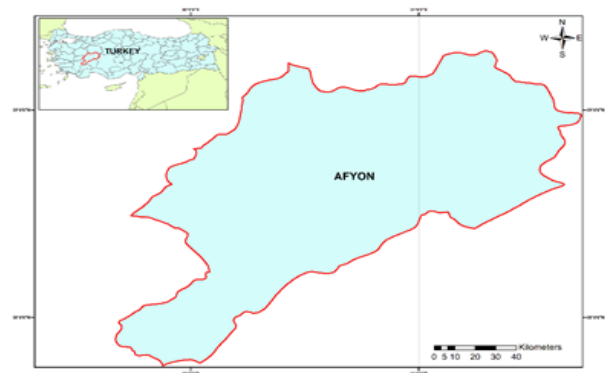


Figure 1. Study area

A typical continental climate is dominant in the study area since it is far from the sea and surrounded by mountains. However, it also exhibits the transition region characteristics. Winters are snowy and cold; summers are hot and dry in the area. In the framework of the epidemiological study, a questionnaire was applied to the owners of sheep in order to learn their point of view on the measurement of MV clinical symptoms in flocks and preventive measures from diseases and to obtain basic knowledge about lamb care, feeding and farm management. Sheep farms were grouped as small, medium and large flocks according to the number of animals.

### Sample collection

A total of 248 blood samples were collected from 22 sheep flocks by the random sampling method. Blood samples placed inside sterile vacuum tubes for serological testing were centrifuged at 3000 rpm for 10 minutes. The obtained sera were taken into stock tubes and stored at -20 °C until testing.

### Detection of MVV Antibodies by ELISA and statistical methods

The commercially available indirect ELISA test kit (MVV/CAEV Test, IDEXX, USA) was used for the detection of MVV-specific antibodies in blood sera. The ELISA test was performed according to the procedure stated by the manufacturer.



The measurement was performed at 450 nm to obtain the Optical Density (OD) data, and the OD data were calculated. Univariate logistic regression analysis was used in the analysis of the data obtained and whether there is a relationship between the relevant variables related to the disease was investigated. Basic descriptive statistics and logistic regression analysis were performed by using R program (R Core Team 2018).

## Results

Out of 248 sheep sera tested, 14 (5.65%) were positive for MVV antibodies. Seroprevalence of infection was detected in 3 of 22 sheep breeding farms (13.63%), while seroprevalence in each flock was detected to be MVV positivity ranged from 2.78% to 8.97%. Seropositivity rates were found to be 3.94% and 13.33% in females and males, respectively. This result statistically significant ( $p < 0.05$ ).

Age-related MV seroprevalence shows that it is different in three age groups. Positivity proportion showed a regular increase by age. (<12 month, %2.2; 12-36 month, %3.5; >36 month, %8.4). On the other hand, MV seroprevalence related to presence of goats shows a significant relationship. The presence of goats in flocks has risk of developing the disease approximately 15 times (OR:15.20 95% CI:3.6-62.55,  $p=0.00001$ ).

The details and results of the distribution of variables including flock size, age, gender and presence of goats in the flock are shown in Table 1. As can be seen in the Table 1, a significant relationship was found between seropositivity of the disease and the presence of goats in gender and flock ( $p < 0.05$ ).

## Discussion

MVV is one of the diseases with economic importance in sheep breeding. MV causes a disease which is characterized by horizontal and vertical infection with a slow progression in the brain, lungs, joints and mammary glands of infected sheep and a lifelong persistence in adult animals. Restrictions on animal movements and bans on international sheep trade are other important problems.

In the eradication of MVV infection, the detection of prevalence is shown to be the best way to maintain the flock with seronegative animals following the separation of seropositive ones from the flock to reduce the high seroprevalence (Peterhans et al 2004). In Turkey, MVV seroprevalence in sheep was reported to be between 1.2% and 41.2% in previous studies (Gürçay and Parmaksız 2013, Un et al 2018). In the past years, the serological data related to MVV differ in other geographical regions of Turkey. In this study, epidemiological research was conducted to analyze the risk factors against MVV for the first time in the Afyonkarahisar province and general seroprevalence was determined to be 5.65% on the basis of individuals and 13.63% on the basis of flocks. Changes in prevalence depend on many factors, such as number of animals, time frame and unsupervised animal movements. Factors such as animal density, reinfection, and the number of infected animals in the initial flock can explain these results. Furthermore, differences in the virus strain generation system may contribute to MVV infection. The reasons such as uncontrollable animal movements play a major role in the spread of viral diseases in different spatial areas. Unsupervised animal movement is one of the reasons for the spread of the diseases worldwide.

Table 1. Values with univariate logistic regression for investigated risk factors for MVV in Afyonkarahisar

Factors	Factor Levels	Number	Positive	Negative	$\chi^2$	p-value	Odds Ratio	95%CI	p-Value
Flock Size	Small *	36	1	35	3.69	0.15	0.28	0.03-2.44	0.25
	Medium	78	7	71					
	Large	134	6	128					
	total	248							
Age	<12 month *	45	1	44	9.56	0.18	0.61	0.06-6.07	0.67
	12-36 month	84	3	81					
	>36 month	119	10	109					
	total	248							
Sex	Male*	45	6	39	5.93	0.01	3.75	1.23-11.41	0.01
	Female	203	8	192					
	total	248							
Presence of goats	Yes*	10	4	6	23.08	0.00001	15.20	3.6-62.55	0.0001
	No	238	10	228					
	total	248							

\*Reference





In some of the farmers in the region, the fact that they exchange animals between farms and there is no health control or serological test may cause the widespread of the diseases within the country. Some of the analyzed farms had limited space and poor ventilation, poor feed quality. These situations support the spread of disease within the flock. Other researchers also point to similar situations (Straub 2004, Hüttner et al 2010, Leginagoikoa et al 2010, Barquero et al 2013, Junkuszew et al 2016, Michiels et al 2018).

In animals, seropositivity rate increased with increasing age as directly proportional. In this study, MVV seroprevalence was found to be 2.22% under the age of 1 year and 3.57% between the ages of 1 and 3 years in a tendency to increase. It was found out that the prevalence increased to three times (8.4%) from the age of 3 years. These findings are similar to the findings of other studies (Okada and Yonemichi 1982, Simard and Morley 1991, Cutlip et al 1992, Arsenault et al 2003, Alba et al 2008, Hüttner et al 2010, Giangaspero et al 2011). This may be explained by the fact that MVV infection may cause life-long infection as a persistent and carrier after infection with MVV based on the age of sheep and with the increased likelihood of exposure to risks. MVV seroprevalence was found to be high and significant in males (13.33%) ( $P=0.00001$ ). As reported in other studies on gender (Simard and Morley 1991, Yavru et al 2012).

The second aim of this study was to analyze and evaluate the risk factors for MVV infection. This study has several limitations to be considered when interpreting its results. The first one is that it may make it difficult to define the relatively small sample size and real flock factors related to MVV infection at the farm level. In the study, it was observed that gender and presence of goats in the flock had as substantial effect as risk factors. Other researchers have reported similar findings in their studies on risk factors and seroprevalence (Shuaib et al 2010, Barquero et al 2013, Kaba et al 2013, Hüttner et al 2017, Michiels et al 2018).

Although the main host of MVV infection is sheep, the virus is also pathogen for goats. MVV seroprevalence was found to be statistically significant in terms of presence of goats. This is difficult to explain and this may be related to flock management factors. It can be described as a less manageable situation in terms of feeding, sheltering and control of the flock. It is thought that there will be cross-species contamination in flocks where sheep and goats are raised together. The researchers reported similar findings in the previous studies (Dawson 1980, Shah et al 2004, Pisoni et al 2005). In order to minimize the spread of the virus in large flocks and reduce seroprevalence, it may be considered to take precautionary measures such as separating the infected and non-infected animals, keeping the new animals of the heard under observation until they are tested and avoiding adjoining infected animals.

## Conclusion

In conclusion, this study provides an overview of the seroprevalence of MVV infection in sheep in Afyonkarahisar province and whether the risk factors investigated have an effect on the disease. Therefore, further studies should be performed in terms of epidemiology of MV disease. Periodic screening of MVV infection is thought to be beneficial for the economic sustainability of the enterprise in order to prevent spread of the disease on a flock basis.

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## Conflict of Interest

The authors did not report any conflict of interest or financial support.

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