



Serological Investigation of Maedi-Visna in Sheep with Chronic Respiratory Disease in Erbil, Iraq*

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Abstract: Maedi-Visna is a chronic and progressive viral disease most commonly seen in adult sheep. Disease is characterized by progressive interstitial pneumonia and/or neurological manifestations. The aim of this study is to serologically reveal the presence of Maedi-Visna in adult sheep in Erbil province in Iraq, which is an important area of sheep farming and also to investigate the prevalence of Maedi-Visna disease in sheep with chronic respiratory problems. Blood samples were obtained from 100 male and female Awassi sheep between 2 and 8 years old which were suffering from chronic pneumonia. The samples were obtained from the different locations in the city of Erbil and its provinces (Hawler, Dashti Hawler, Maxmur and Xebat) between January and April 2016. Blood samples were analysed for Maedi-Visna by using ELISA method in laboratory. According to the analysis, of 100 samples; positive, suspected and negative results were determined as 65%, 3% and 32%, respectively. Seropositivity increased with regard to age and it was determined higher in ewes. Highest seropositivity was determined in Hawler. Clinical findings such as emaciation, coughing, dyspnea, mastitis and paralysis were detected considerably high in the animals. As a result, Maedi-Visna was observed in animals with chronic respiratory system disease with high rate in Iraq-Erbil region. As this is the first study performed in the region related with Maedi-Visna, we believe that this study will provide a basis for the further studies in the region.

Keywords: ELISA, Maedi-Visna Virus, Pneumonia, Seroprevalence, Sheep.

Irak, Erbil Bölgesinde Kronik Solunum Sistemi Hastalığı Olan Koyunlarda Maedi-Visna Hastalığının Serolojik Araştırılması

Öz: Maedi-Visna erişkin koyunlarda yaygın bir şekilde görülen, kronik ve progresif bir viral hastalıktır. Hastalık progresif interstisyel pnömoni ve/veya sinirsel semptomlarla karakterizedir. Bu çalışmanın amacı, Irak-Erbil bölgesinde kronik solunum sistemi problemi olan erişkin koyunlarda Maedi-Visna hastalığının ELISA yöntemiyle prevalansının ortaya konulmasıdır. Kronik pnömonili, 2-8 yaş arası, dişi ve erkek 100 İvesi ırkı koyundan kan örnekleri toplandı. Örnekler Erbil ilinin değişik bölgelerinden (Hawler, Dashti Hawler, Maxmur ve Xebat), Ocak ve Nisan 2016 ayları arasında toplandı. Kan örnekleri laboratuvarında ELISA yöntemine göre Maedi-Visna hastalığı yönünden analiz edildi. Yapılan analizler sonucu, 100 örnekten 65'i pozitif, 3'ü şüpheli ve 32'si negatif olarak tespit edildi. Hastalığın seropozitifliğinin yaşla birlikte artış gösterdiği ve dişilerde daha yüksek oranda olduğu gözlemlendi. Bölgelere göre en yüksek seropozitiflik ise Hawler'de belirlendi. Klinik bulgu olarak, hayvanlarda aşırı kilo kaybı, öksürük, dispne, mastitis ve paralizis yüksek oranda belirlendi. Sonuç olarak, Irak-Erbil bölgesinde kronik solunum sistemi hastalıklı hayvanlarda Maedi-Visna yüksek oranda tespit edildi. Bu çalışmanın bölgede Maedi-Visna hastalığı ile ilgili yapılan ilk çalışma olduğu ve gelecekte yapılacak çalışmalara ışık tutacağı kanısına varıldı.

Anahtar Kelimeler: ELISA, Koyun, Maedi-Visna Virüs, Pnömoni, Seroprevalans.

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*This research is summarized in the same master's thesis.

INTRODUCTION

Maedi-Visna is caused by small ruminant lentiviruses (SRLV) within the genus lentivirus belonging to family Retroviridae. Maedi-Visna is a chronic and progressive viral disease most commonly seen in adult sheep and the disease is characterized by progressive interstitial pneumonia or neurological manifestations (1).

There are two forms of the disease. Maedi is the respiratory form and Visna is the nervous form (2,3). Maedi-Visna leads to multi-systemic inflammatory disease. The four major tissues affected are the lungs, mammary glands, joints and CNS. However, pathological lesions can also be seen in other organs including heart, liver, kidney lymph nodes, blood vessels and bone marrow. MVV has a very long incubation period and the clinical signs do not appear until the age of 2 years and many sheep remain asymptomatic carriers for their whole lives (2,4-6).

The clinical signs of Maedi are observed more common than Visna. The clinical signs of Maedi are coughing, dyspnea, emaciation and mastitis. Abortions are also recorded but associated with severity of infection (1,5,7,8). Clinical signs of Visna are weakness in the hind legs, arthritis, weight loss, mastitis and progression to complete paralysis and sometimes CNS disorders are also observed. In both Maedi and Visna, the body temperature does not increase if there is not a secondary infection (4,8).

Diagnosis of MVV infection can be made by clinical findings and the confirmative diagnosis must be performed by laboratory methods such as post-mortem examination, histopathological lesions, virus serology and isolation. Several methods are used for serological diagnosis of MVV including AGID, ELISA, RIA, RIPA and WB and PCR (6,7,9).

Direct or indirect transmission occur by contact with lung secretions and excretions of infected sheep. There is no effective treatment and no available vaccine. Supportive treatment that includes suitable management and control of secondary

infection with antibiotics may lengthen life for a few weeks or months but the disease is finally fatal. Because of the poor prognosis and risk of exposure of uninfected animals to clinical disease, long-term treatment is not recommended (2,7).

In the present, Maedi-Visna spreads sheep on the entire world in most European countries and recognized in several continents such as USA, Africa and Asia where the disease caused important economic losses. Very few countries are free of MVV like New Zealand and Australia. In many countries, MV was not described and investigated until now such as Algeria, Egypt, Lebanon, Libya and Tunisia (3,5,10). In Iraq, also there is not any report about this disease.

The aim of this study is to serologically reveal the presence of Maedi-Visna in adult sheep in Erbil province in Iraq, which is an important area of sheep farming and also to investigate the prevalence of Maedi-Visna disease in sheep with chronic respiratory problems.

MATERIALS and METHODS

Blood samples were obtained from 100 male and female Awassi breed sheep between 2 and 8 years old, which were suffering from chronic pneumonia. The samples were obtained from the different locations in the city of Erbil (Hawler, Dashti Hawler, Maxmur and Xebat) and its provinces between January and April 2016. This research was approved (28. 01. 2016 and 2016/01) by the Animal Research Ethics Committee of Yuzuncu Yil University in Van, Turkey.

Following clinical examination; obtained clinical findings such as sampling date, address, animal breed, clinical examination (age, body temperature, heart rate and respiratory rate) and clinical signs (coughing, dyspnea, paralysis, emaciation, mastitis and arthritis) were recorded.

For this purpose, 10 ml of blood was taken from the jugular vein and kept in the non-anticoagulant tube. Then, blood samples were transported to the

Erbil veterinary laboratory in cold chain within the hours of collection and the blood was centrifuged at 3000 RPM for 15 minutes to extract serum. Each serum of samples was divided into 3 eppendorf tubes and stored in a deep freezer at -20°C. Samples were transferred to Internal medicine laboratory, Veterinary Faculty, Yuzuncu Yil University in cold chain in order to perform analyses.

A commercial ELISA kit (CAEV/MVV total Antibody Test Kit, IDEXX/Switzerland 06-41199-01) was used for detecting antibodies against MVV in the serum. All serum samples were analyzed according to the procedure provided by the company. The results were read by using (ELISA reader® DAS) at 450 NM wavelength and obtained absorbency values were calculated according to the protocol provided by the kit. S/P ratio under 50% was considered as negative, between 50-60% was considered suspect and over 60% was considered as positive.

Statistical Analysis

In this study, evaluation of statistical analysis was performed by using SPSS package program.

Study data was subjected to normality test (Kalmagrov Simirnov). Since this data shows a normal distribution, parametric methods were used for statistical analysis. ELISA results and percentages of obtained results were calculated in terms of sex, age, region and clinical findings. Besides, statistical comparison of body temperature, respiratory rate and heart rate belong to positive, suspected and negative animal groups were performed by using One-Way ANOVA test.

RESULTS

Clinical Findings

General clinical examination of animals with chronical respiratory system symptoms were performed and obtained clinical findings were recorded. MV disease positive, suspected and negative samples according to ELISA results and Xmin, Xmax and Mean±SEM values belong to these clinical findings (body temperature, respiratory rate and heart rate) were given on table 1.

Table 1. Body temperature, respiration and heart rates of positive, suspected and negative animal groups according to ELISA results.

Tablo 1. ELISA sonuçlarına göre pozitif, şüpheli ve negatif hayvan gruplarının vücut sıcaklığı, solunum ve kalp frekansları.

Clinical findings	ELISA Results	X _{min}	X _{max}	Mean±SEM	Significant
Body Temperature (°C)	<i>Positive</i>	38.3	42.3	39.8 ± 0.1	
	<i>Suspected</i>	39.4	40.3	39.7 ± 0.3	
	<i>Negative</i>	38.4	42.2	39.5 ± 0.2	
	<i>Total</i>	38	42	39.2 ± 0.1	0.409
Respiratory Rate (/Minute)	<i>Positive</i>	29	44	37.6 ± 0.5	
	<i>Suspected</i>	36	43	40.0 ± 2.1	
	<i>Negative</i>	27	42	36.3 ± 0.6	
	<i>Total</i>	27	44	37.3 ± 0.4	0.106
Heart Rate (/Minute)	<i>Positive</i>	80	98	87.2 ± 0.6	
	<i>Suspected</i>	80	98	94.0 ± 3.0	
	<i>Negative</i>	80	98	88.2 ± 0.7	
	<i>Total</i>	80	98	87.7 ± 0.5	0.032

P<0.05 values were accepted statistically significant.

Serological Findings

Serum samples were analysed by using ELISA. According to the analysis, of 100 samples; positive, suspected and negative results were determined as 65 (65%), 3 (3%) and 32 (32%), respectively.

Findings According to Sex

100 blood samples were taken randomly from 95 female and 5 male animals. While 63 of 95 samples (66.3%) taken from female animals were seropositive, 3 samples were suspected and 29

samples were negative. While 2 of 5 samples (40%) taken from male animals were seropositive, 3 samples were negative and there was not any suspected sample.

Findings According to Age

According to the analysis, seropositivity was not determined in animals with 2 years of age. Seropositivity ratio of other age groups were given on table 2. When table 2 was analysed, seropositivity rate of the disease progress with increasing age.

Table 2. ELISA results and positivity rates according to the ages of sheep.

Tablo 2. Koyunlarda yaşa göre ELISA sonuçları ve pozitiflik oranları.

Years	Number of samples (n: 100)	Positive	Suspected	Negative	Positive Percentage (%)
2	3		1	2	0
3	11	5	1	5	45.5
4	13	7		6	54
5	20	13	1	6	65
6	20	14		6	70
7	18	13		5	72
8	15	13		2	86

Findings According to Regions

In this study, 100 blood samples were taken from animals in Hawler, Dashti Hawler, Maxmur and Xebat in Erbil city. Number of samples and seropositivity rate were given on Table 3. When table

3 were examined, the highest seropositivity rate was determined in Hawler (73.7%). Seropositivity rate in other regions were determined in Dashti Hawler, Maxmur and Xebat as 65.4%, 64.3% and 53.8%, respectively.

Table 3. The prevalence rate of MVV infection in different geographical locations.

Tablo 3. Farklı coğrafi bölgelerdeki MVV enfeksiyonunun prevalansı.

Locations	Number of samples	Positive	Suspected	Negative	Positive Percentage (%)
Hawler	19	14	-	5	73.7
Dashti Hawler	26	17	1	8	65.4
Maxmur	42	27	2	13	64.3
Xebat	13	7	-	6	53.8

Results According to Clinical Findings

Serological datas were given on table 4 in terms of clinical findings such as coughing, dyspnea, paralysis, emaciation, mastitis and arthritis. According to these findings; percentage of

emaciation, coughing, dyspnea, mastitis and paralysis were as 95.3%, 90.7%, 75.3%, 10.7% and 6.1%, respectively. However, none of the animals in this study showed symptoms of arthritis (Table 4).

Table 4. Results of clinical signs of all samples.

Tablo 4. Tüm örneklerin klinik bulgularının sonuçları.

Clinical sign	Positive/ Showing clinical sign	Suspected/ Showing clinical sign	Negative/ Showing clinical sign	Positive percentages (%)
Emaciation	62/65	3/3	31/32	95.3
Coughing	59/65	2/3	25/32	90.7
Dyspnea	49/65	3/3	25/32	75.3
Mastitis	7/65	0/3	0/32	10.7
Paralysis	4/65	0/3	2/32	6.1
Arthritis	0/65	0/3	1/32	0

DISCUSSION and CONCLUSION

Maedi-Visna is a chronic and progressive viral disease most commonly seen in adult sheep and the disease is characterized by progressive interstitial pneumonia or neurological manifestations (1). The aim of this study is to reveal the presence of Maedi-Visna serologically in adult sheep in Erbil province in Iraq, which is an important area of sheep farming and also to investigate the prevalence of Maedi-Visna disease in sheep with chronic respiratory problems.

In previous studies, it was reported that Awassi sheep are sensitive to Maedi-Visna disease and it leads to infection in this breed widely. The disease is commonly observed in worldwide except New Zealand and Australia, and causes important economic losses (3,5,10,11). Several studies were performed in different regions of the world. Incidence of the disease in Poland, Austria, Canada, USA, Morocco, Finland and Switzerland was reported as 24%, 9.5%, 19%, 26%, 24.8%, 1.6% and 9%, respectively (12-18). In many countries, MV was not described and investigated until now such as Algeria, Egypt, Lebanon, Libya and Tunisia (5). In Iraq, also there is not any report about this disease.

In a study related with Awassi sheep, Seyoum et al. (19) reported seroprevalence of the disease as 38%. In another study (20), seroprevalence of the disease in Awassi sheep was reported as 6% in Syria. Norouzi et al. (21) reported seroprevalance of the disease as 34.5% in Iran. In studies performed in Turkey, seroprevalence of the disease was determined as 6.45% in Van (3), 19.4% in Kirikkale (22) and 21.1% in Şanlıurfa (10).

According to literature research, the disease was encountered to a high degree in neighbour countries of Iraq (3,10,20-22) however, there was not any study found related with the presence of disease in Erbil region, Iraq. In the current study; according to ELISA results, while 65 of 100 animals were seropositive (65%), 3 of 100 were suspected (3%) and 32 of 100 were seronegative (32%). When compared with other studies (3,10,20-22), high seropositivity was determined in our study. Because the blood samples were only obtained from animals with chronic respiratory diseases. Besides, detecting Maedi-Visna disease in animals with chronic respiratory system problems may indicate that the disease is common in the region.

In most of previous studies (14,21,23), the disease was detected in female animals more than the males. While Seyoum et al. (19) reported the seropositivity higher in male animals than females, Cutlip et al. (15) reported that there was not any statistical significance between male and female animals. In our study, seropositivity was also determined higher in female animals (66.3%) than male animals (40%).

In a study, seropositivity was reported higher in adult sheep (72.89%) than young sheep (23). In previous studies, it was also reported that seropositivity of the disease increased with age (14,24). However, Simard and Morley (14), reported that the prevalence of the disease decrease in 9 years old animals. Contrary, Lamontagne et al. (1) determined that seropositivity rates were not statistically significant in terms of age. In the present study, while none of any 2 years old animals had seropositivity, seropositivity increased in animals gradually with age. However, while seropositivity was determined in 2 years old sheep (14,15,23,24), in our study; we did not determine seropositivity in this age group. The reason of increasing seropositivity with age is due to the chronic progress of the disease and long replication period of the virus in host monocytes/macrophages (25).

According to the literature research, none of any study was performed related with Maedi-Visna disease in Erbil, Iraq. Blood samples of this study were obtained from Hawler, Deshti Hawler, Maxmur and Xebat regions in Erbil, Iraq. Seropositivity was determined as 73.7% in Hawler, 65.4% in Dashti Hawler, 64.3% in Maxmur and 53.8% in Xebat. According to these results, the disease was detected with highest prevalence in Hawler and in lowest prevalence in Xebat.

Clinical signs of Maedi occur more commonly than Visna (5,7). The clinical signs of Maedi are coughing, dyspnea, emaciation and mastitis (8). Abortions are also recorded but associated with severity of infection (1). The clinical signs of Visna are weakness in the hind legs, arthritis, weight loss and

mastitis. These signs progress to complete paralysis and sometimes CNS disorders arise. In both Maedi and Visna, body temperature increases only in terminal stage due to secondary infection (4,8). Clinical findings detected in several studies (1,8,24,26) related with Maedi Visna disease comply with findings detected in this study. In our study, clinical findings such as emaciation, coughing, dyspnea, mastitis and paralysis were determined as 95.3%, 90.7%, 75.3%, 10.7% and 6.1%, respectively, in seropositive animals. In our study, percentage of clinical symptoms detected in seropositive animals were found higher than the study performed by Lamontagne et al. (1). We presume the reason of this condition as all of the samples were obtained from adult sheep with chronic pneumonia. Lamontagne et al. (1) reported in their study that emaciation was only detected in animals older than 3 years. Similarly, we also found that emaciation was considerably high in seropositive animals. However, in another study, Benavides et al. (27) did not find any clinical findings such as dyspnea and emaciation and they did not find any findings related with respiratory system.

Besides, in other studies (9,24,26) researchers reported mastitis as a common finding, which is similar to the findings we emphasize in our study. Because in our study, all of the animals with mastitis were seropositive according to ELISA results.

While Sigurdsson et al. (28) and Pritchard et al. (29) reported neurological symptoms in adult sheep, Benavides et al. (27) and Akkoc et al. (30) reported that lambs can also show neurological symptoms. Our study was similar to Sigurdsson et al. (28) and Pritchard et al. (29), we detected neurological symptoms in some of the animals. In our study, one of the animals showed symptoms of arthritis; however, the animal was seronegative according to ELISA result. Despite the fact that Maedi-Visna may be seropositive in animals showing clinical arthritis symptoms (5); in this study, seropositivity was not determined in animals with arthritis.

As a result, Maedi-Visna was seen in animals with chronic respiratory system disease with high

rate in Erbil, Iraq. As this is the first study performed in the region related with Maedi-Visna, it is concluded that further detailed studies should be performed, determining seropositive animals with the support of regional management, control and eradication programs should begin at the earliest opportunity. We believe that this study will provide a basis for the further studies in the region.

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