



## Detection of Maedi-visna virus in sheep in Nineveh province

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### Abstract

This is a primary study in detecting a specific antibody of the Maedi-visna virus (MVV) in blood samples and viral antigen in nasal swabs from sheep. Two hundred and forty nasal swabs and blood samples were collected from sheep of different ages and health statuses in Nineveh province, Iraq. Enzyme-linked immune sorbent assay was used to detect specific antibodies against MVV specific proteins gp135 and p25, Whereas Agar gel immune diffusion (AGID) was used to detect antigens from nasal swabs using specific antibodies against gp135 proteins of MVV. Serological results using ELISA showed the total prevalence of MMV 22.9% and the highest prevalence of infection in sheep less than one-year-old 36.5% when compared with older animals 8.4%. Animals that suffering from respiratory problems had a higher prevalence of infection 25.7% compared with healthy animals 6.9%. AGID showed an identical reaction between Maedi-visna viruses and specific rabbit antisera with a total infection rate of 12.9%. The highest prevalence of infection was 22.4% recorded in animals less than one-year-old, the lowest percentage of infection in animals more than two years of age was 1.4%. Animals suffering from respiratory signs also showed high prevalence of infection 13.8%, while the lowest percentage of infection were recorded in healthy animals 4.6%. It has been concluded that the high prevalence of infection rate in sheep less than one-year-old and the animals which suffered from respiratory problems had higher prevalence according to ELISA and AGID testes.

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### Introduction

Different viruses were affect sheep and goats breeding in the Nineveh governorate, Maedi-visna virus (MVV) is one of that which (a lentivirus that infects sheep and goats) causing encephalitis and arthritis, however is also known as ovine progressive pneumonia (OPP) (1,2). The comparison of nucleic acid sequences of MVV and caprine arthritis encephalitis virus (CAEV) using phylogenetic analysis showed clear indications of cross-species between the two viruses (3). MVV and CAEV are characterized by lifelong persistent infection in host monocytes and macrophages with a long time between the entrance of the virus and the appearance of detectable specific antibodies. The majority

of infected sheep and goats do not manifest clinical signs, but remain persistently infected and capable of transmitting the infection and shedding the virus (4). The clinical signs of these viruses are paralyzing meningoencephalitis, progressive pneumonia (which are the main signs of MVV infection), chronic arthritis, and synovitis (which are the main symptoms of CAEV infection). Encephalitis occurs primarily in newborn animals between 8 and 24 weeks following CAEV infection (5). The agar gel immunodiffusion test (AGID) was used to diagnose infection (6). Indirect and competitive enzyme-linked immune sorbent assays (I-ELISAs and c-ELISA, respectively) were used to determine anti-MVV or anti-CAEV antibodies in the sera of animals (7). A few of the I-

ELISAs showed high sensitivity and specificity against a standard of comparison; one c-ELISA showed high sensitivity and specificity in both sheep and goats in the USA, suggesting that this one test could be used for both MVV and CAE (8).

## Materials and methods

### Animals and samples collection

Two hundred forty sheep of different ages, and breeds reared in different regions in Nineveh province, Iraq were studied herein. Some of the animals were suffering from nasal discharge, loss of appetite, cough, and dyspnea, while others appeared clinically healthy. Blood samples and nasal swabs were collected from each animal. Blood samples were clotted after being placed in 25°C temperature for 15 minutes. Then the serum was separated by centrifuging at 1500 rpm for 10 minutes and stored at -20°C (9). The nasal swabs were collected and then added to 5 ml of sterile phosphate buffer saline to a glass tube and centrifuged at 6000 rpm for 1 hour. The sediment was discarded and the supernatant was stored at -20°C (10).

### Enzyme-linked immune sorbent Assay (in direct ELISA)

I-ELISA (ID. Vet. VISNAS -5P) was used according to the manufacturer's instructions to investigate the specific IgG immunoglobulin against peptides from the MVV/CAEV TM, gp135 and p 25 proteins

### Agar gel diffusion

The agar gel diffusion test was performed as double diffusion in agar gel according to (11) to detect viral antigens in nasal swabs using specific antisera. Agar gel immunodiffusion test kits for diagnosing Maedi-Visna (RAI0260) in sheep consisted of precipitating antiserum to the glycosylated envelope polypeptide (gp 135) antigen of MVV.

Table 2: Percentage of infection with Maedi-visna virus by using ELISA according to health status of the animals

Health status	Number of blood samples	Number of positive samples (%)
Suffering from respiratory symptoms	167	43(25.7) <sup>a</sup>
Clinically healthy animals	173	12(6.9) <sup>b</sup>
Total	240	55(22.9)

Different letter were significantly different at P<0.05.

Table 3: Percentage of infection with Maedi-visna virus by using AGID according to age of the animals

Age	Number of nasal swabs samples	Number of positive samples(%)
Less than 1 year	107	24(22.4) <sup>a</sup>
1 -2 years	62	5(8.1) <sup>b</sup>
More than 2 years	71	1(1.4) <sup>c,b</sup>
Total	240	31(12.9)

Different letter were significantly different at P<0.05.

## Statistical analysis

The variations in the prevalence of MVV between the ages of animals and the animal's status were evaluated by employing two-sided Chi-square and Fischer's exact test in IBM-SPSS statistics version19 program.

## Results

The result of serological investigations of Maedi-visna virus antibodies using ELISA test showed 22.9% prevalence of infection with Maedi-visna virus. The prevalence of infection was higher in sheep less than one-year-old 36.5% when compared with older sheep 8.4% (Table 1). A higher prevalence of infection was recorded in animals suffering from respiratory symptoms 25.7% when compared with healthy animals 6.9% (Table 2). The results of the agar gel diffusion analysis showed identical positive reaction between Maedi-visna viruses and specific rabbit antisera. The percentage of total infection rate was 12.9%, and animals less than one year of age showed the highest rate of infection 22.4%, and the lowest percentage rate of infection was recorded in animals more than 2 years old 1.4% (Table 3). Results of the agar gel diffusion analysis showed a higher prevalence of infection in animals with respiratory signs 13.8%, while the lowest percentage of infection was recorded in healthy animals 4.6% (Table 4).

Table 1: Percentage of infection with Maedi-visna virus according to age of the animals using ELISA

Age	No. blood samples	No. positive (%)
> 1 year	107	39 (36.5) <sup>a</sup>
1 -2 years	62	10 (16.7) <sup>b</sup>
< 2 years	71	6 (8.4) <sup>c,b</sup>
Total	240	55 (22.9)

Different letter were significantly different at P<0.05.

Table 4: percentage of infection with Maedi-visna virus by using AGID according to health status of the animals

Health status	Number of nasal swabs samples	Number of positive samples (%)
animals suffering from respiratory symptoms	167	23(13.8) <sup>a</sup>
Clinically healthy animals	173	8(4.6) <sup>b</sup>
Total	240	31(12.9)

Different latter were significantly different at  $P < 0.05$ .

## Discussion

The detection of Maedi-visna virus antibodies in sheep confirms the infection. The virus can be replicated in cells which may be restricted, thus limiting viral antigen production. Limited antigen expression will not induce a good immunological reaction, and some animals may stay give ELISA-negative, despite carrying the virus being virus positive (12). The results of the current study showed a high prevalence of infection in young animals, while several studies that aimed to determine the relationship between infection rate and animal age, Kita *et al.*, (13) recorded a high prevalence of infection in adult sheep more than 5 years old. The difference in prevalence of infection among different ages was seen but not proven because of inadequate numbers, while Ernst *et al* (14) reported an infection rate of 18% in sheep less than 2 years old and Giangaspero *et al* (15) reported 6% total infection rate in Syrian Awassi sheep. Varea *et al* (16) indicated a high prevalence of infection in sheep up to 3 years old when compared with sheep less than a year old. Luqman and Cumali (5) recorded a high prevalence of infection in adult animals in Erbil province, Iraq. The differences among studies can be explained by the differences in animal management methods, the sources of the animals (local or imported breeds), and the presence of stress factors like shipping or the presence of a secondary infection.

The highest prevalence rate of infection was recorded in animals suffering from respiratory symptoms when compared with healthy animals, in line with the results of many other researchers. Luqman and Cumali (5) recorded a high prevalence of Maedi-visna virus in animals suffering from respiratory symptoms. Esperanza *et al* (17) showed a relationship between respiratory and other clinical signs and Maedi-visna seropositivity. The agar gel diffusion results showed an identical reaction between Maedi-visna viruses and specific antisera. The total infection rate was 12.9%, and animals younger than one-year-old and those suffering from respiratory symptoms showed the highest infective rates. Agar gel diffusion has been used by several researchers with great success in detecting viral antigens and antibodies; for example, Alluwaimi *et al* (18), who used AGID to detect viral antibodies in Saudi Arabian sheep. Varea *et al* (16) used ELISA and AGID in their comparative study of these techniques to detect viral antibodies. Carole *et al* (19) successfully compared ELISA and AGID in detecting viral antibodies. Other researchers

successfully investigated viral antigens in lymphoid tissue in sheep suffering from respiratory signs by using another technique (PCR, IHC) (20), while Reyburn *et al* (21) used P24 antigens as a method to detect viral antigens. The presence of seropositive sheep within herds can be explained by the importation of feedlot sheep, poor quarantine measures, the lack of monitoring of sheep flocks to and from unobserved fringe seropositive Maedi-visna virus polities, and contact between unhealthy and healthy animals. This study was conducted in the presence of Maedi-visna virus infection in the polities adjoining Iraq (Iran, Turkey, and Syria) (4,22).

## Conclusions

Using ELISA test to detect antibodies in serum samples against MVV and AGID to detect viral antigen in nasal swabs showed high prevalence of infection rates in sheep less than one-year-old and animals suffering from respiratory problems.

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## Conflict of interest

The Authors declare no conflict of interests of the manuscript.

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## الكشف عن فايروس Maedi visna في الأغنام في محافظة نينوى

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### الخلاصة

تعد هذه دراسة من الدراسات الأولية في الكشف عن الأجسام المضادة المتخصصة لفيروس Maedi visna في عينات الدم والمستضد الفيروسي في مسحات الأنف للأغنام. تم جمع مائتين وأربعين مسحة أنف وعينة دم من أغنام من مختلف الأعمار والحالات الصحية في محافظة نينوى، العراق. تم استخدام اختبار الممتز المناعي المرتبطة بالإنزيم للكشف عن الأجسام المضادة المتخصصة للبروتين السكري 135، والبروتين 25 بينما تم استخدام اختبار الانتشار المناعي على هلامة الاكار للكشف عن المستضدات من مسحات الأنف باستخدام أجسام مضادة متخصصة للبروتين السكري 135، بينت نتائج الاختبار المصلي نسبة إصابة كلية بلغت 22,9% وأعلى معدل انتشار للإصابة في الأغنام للأعمار أقل من سنة 36,5% بالمقارنة مع الحيوانات الأكبر سنا 8,4%. أظهرت الحيوانات التي تعاني من مشاكل في الجهاز التنفسي معدل انتشار أعلى للإصابة 25,7% مقارنة بالحيوانات السليمة 6,9%، وأظهرت تفاعلاً متطابقاً بين الفيروسات ومضادات الأرانب النوعية المتخصصة بنسبة إصابة إجمالية قدرها 12,9%. سجلت أعلى نسبة إصابة بالحيوانات أقل من سنة واحدة بنسبة 22,4%، بينما سجلت أقل نسبة إصابة في الحيوانات التي تجاوزت السنتين من العمر 4,1%. أظهرت الحيوانات التي تعاني من أعراض تنفسية انتشار العدوى 13,8%، بينما سجلت أقل نسبة إصابة في الحيوانات السليمة 4,6%.