Iranian Journal of Virology 2010;4(1): 29-33
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Short Communication

Serological study of Maedi-Visna virus among sheep flocks in Kerman province of Iran

Sakhaee E[1*], Khalili M[2]

1. Department of Clinical Sciences, Faculty of Veterinary Medicine, Shahid Bahonar University of Kerman, Kerman, Iran.
2. Department of Pathobiology, Faculty of Veterinary Medicine, Shahid Bahonar University of Kerman, Kerman, Iran.

Maedi-Visna is a viral disease of sheep caused by a lentivirus. It is characterized by progressive interstitial pneumonia or meningoencephalitis. Sometimes arthritis, ill-thrift and indurative mastitis are observed. The disease most often progresses slowly, but irreversibly. The disease occurs in all major sheep producing countries with the exception of Australia, New Zealand, and Finland. The international movement of sheep has facilitated the spread of the disease. Sheep and goats are the only species known to be susceptible (1). All breeds of sheep appear to be susceptible to infection, but there may be differences in breed susceptibility in seroprevalence in flocks with more than one breed of sheep (2, 3). The prevalence of infection varies between farms, and countries. Rates of seropositivity increase with age, and flock seroprevalence is influenced by the average age of the flock[1]. Flock seroprevalence also has been positively associated with the use of foster ewes, allowing lambs older than 1 day to have contact with other lambing ewes, flock size, close contact during confinement for lambing stocking density on pasture, and the length of time that the flock has been in existence (4, 5). Rates of seropositivity are much higher in flocks that also are infected with pulmonary adenomatosis than those which are not. The disease is spread by the respiratory route, ingestion of infected milk and in utero infection. The relative importance of these routes appears to vary with the flock and its management, but lateral transmission is important in all. Economic importance of the disease rests with losses associated with decreased longevity and mortality with clinical disease, decreased value of cull animals and possible effects of sub-clinical infection on productivity (1).

As no treatment or vaccine is available at the present time for the disease, the eradication programs are based on early detection of the disease accompanied by elimination of the animals carrying the virus. This involves detection and culling of seropositive animals where lateral transmission is the dominant mode of transmission in the flock. All sheep on the farm are serologically tested annually or twice a year, and seropositive animals and their progeny of less than 1 year of age are culled and kept separately from the seronegative ones. Flock status with respect to the presence or absence of infection and the determination of the infection status of an individual sheep currently relies on serological testing. Agar gel immunodiffusion (AGID) tests and Enzyme-linked immunosorbent assay (ELISA) tests are used in most countries. Indirect ELISA and competitive ELISA tests may have better sensitivity, depending on the antigen used (6, 7). The value of serological testing with current methodologies rests primarily with the establishment of the infection status of the flock. A negative test in an individual sheep

***Corresponding author:** Ehsanollah Sakhaee (DV.M, Ph.D), Department of Clinical Sciences, Faculty of Veterinary Medicine, Shahid Bahonar University of Kerman, Kerman, Iran.
Tel: (+98) 341 32 02 926
Fax: (+98) 341 32 22 047
Email: Ehsan_sakhaee@yahoo.com

[Downloaded from journal.isv.org.ir on 2026-11-02]
[DOI: 10.21859/isv.4.1.29]

could mean that the sheep is free of infection, but can also occur in an infected animal that has not yet responded to infection (1).

To the authors' knowledge, that there are very few reports of Maedi-Visna in Iran and these are old histopathologic reports. The aim of this study was to investigate the presence of antibodies against Maedi-Visna virus among sheep flocks by indirect ELISA in Iran.

A total of 138 serum samples were collected from twelve sheep flocks in Kerman, (three flocks from Kerman district and one flock from other districts of Kerman province) from September 2009 to April 2010. The flocks were selected randomly, and about 10% of the population in each herd was tested. Blood samples were taken aseptically using a sterile 10-ml anticoagulant-free vacutainers from jugular vein. Samples were transported on ice to the laboratory, and sera were separated immediately by centrifugation of blood at 1500×g for 10 min at room temperature and were kept at −20°C until the day of analysis.

Serum samples were tested for the presence of antibodies against Maedi-Visna virus using the indirect ELISA kit (Pourquier®, France) and coated plates with the immunogenic peptide of a trans-membrane protein (TM, ENV gene) of the recombinant P28 protein, part of the viral capsid (GaG gene). The appearance of anti-P28 antibodies can occur slightly later than that of the anti-viral envelope protein antibodies. Sera were prepared at 1:20 dilution, and specific antibodies were measured using a peroxidase-labeled anti-ruminant immunog-lobulin G (IgG) conjugate. Results were expressed as the optical densities at 450 nm reading of the test sample (value), calculated as subtraction the OD 450 value obtained from the uncoated well from the OD 450 value from the coated well. The results were considered reliable if: the positive control serum had a minimal mean uncorrected OD 450 value of 0.350, and a ratio between the mean corrected OD 450 value of the positive control and corrected OD 450 value 0f the negative control was greater than or equal to 3.5.

According to Table 1, antibodies were detected in 3 sera (2.175 %) of 138 samples. One male (0.725 %) and two female (1.45 %) cases had antibodies against to Maedi-Visna virus. Positive serum samples were found in flocks 2 (0.725 %), 3 (0.725 %), and 9 (0.725 %). 135 cases (97.82 %) out of 138 sheep were negative.

This is the first study determining seroprevalence of the disease in Kerman province of Iran. In the present study, the seroprevalence of Maedi-Visna virus infection was 2.175 %. This result was in accordance to the previously reported by Sayari and Lotfi (2001) who found that 0.54 % from total inspected lungs had Maedi like lesions. Lower seroprevalence of lentiviruses infection has been reported using ELISA test in UK 4.3% (9), Switzerland 2%[10], Turkey 1.9% (11), Mexico, 0.4% [12], and Italy 4.2% (13).

The detection of antibodies against Maedi-Visna virus in sheep is considered to be an evidence for infection[14]. However, virus replication in infected cells may be restricted, thus limiting viral antigen production. Limited antigen expression will not induce a fierce immune response, and some animals may remain ELISA-negative, in spite of being virus positive. Delayed seroconversion, i.e., seroconversion later than 6–12 weeks after an infection, has been well documented for lentiviruses (15-19).

It is suggested that uncontrolled movement of sheep flocks from and to uncontrolled border countries and contact with other herds could explain presence of seropositive sheep within examined herds. This study also suggested the possible presence of Maedi-Visna virus infection in the neighboring countries of Iran, as some of the positive reactors were imported from those countries. Further studies are needed to confirm this finding. The low seroprevalence of Maedi-Visna virus infection in Kerman, Iran, indicates the importance of establishing measures to prevent the entrance of more positive reactors into the country.

The information obtained on seroprevalence in this study is considered an important initial step towards the establishment of further control and eradication measures. Prevention of Maedi-Visna virus infection is important in sheep flocks because there is no treatment to eliminate Maedi-Visna virus or a vaccine to

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[DOI: 10.21859/isv.4.1.29]

Table 1. Prevalence of antibodies specific to Maedi-Visna virus in 12 sheep flocks in Kerman, Iran.

Flocks	Total sera			Positive sera			Negative sera		
	Male	Female	Total	Male	Female	Total	Male	Female	Total
Flock 1	4 (2.90)[a]	6 (4.35)	10 (7.25)	0 (0.00)	0 (0.00)	0 (0.00)	4 (2.90)	6 (4.35)	10 (7.25)
Flock 2	10 (7.25)	8 (5.79)	18 (13.04)	1 (0.725)	0 (0.00)	1 (0.725)	9 (6.52)	8 (5.79)	17 (12.31)
Flock 3	5 (3.62)	4 (2.90)	9 (6.52)	0 (0.00)	1 (0.725)	1 (0.725)	5 (3.625)	3 (2.175)	8 (5.80)
Flock 4	3 (2.17)	4 (2.90)	7 (5.07)	0 (0.00)	0 (0.00)	0 (0.00)	3 (2.17)	4 (2.90)	7 (5.07)
Flock 5	5 (3.625)	5 (3.625)	10 (7.25)	0 (0.00)	0 (0.00)	0 (0.00)	5 (3.625)	5 (3.625)	10 (7.25)
Flock 6	6 (4.35)	10 (7.25)	16 (11.60)	0 (0.00)	0 (0.00)	0 (0.00)	6 (4.35)	10 (7.25)	16 (11.60)
Flock 7	5 (3.625)	3 (2.175)	8 (5.80)	0 (0.00)	0 (0.00)	0 (0.00)	5 (3.625)	3 (2.175)	8 (5.80)
Flock 8	5 (3.625)	5 (3.625)	10 (7.25)	0 (0.00)	0 (0.00)	0 (0.00)	5 (3.625)	5 (3.625)	10 (7.25)
Flock 9	6 (4.35)	8 (5.80)	14 (10.15)	0 (0.00)	1 (0.725)	1 (0.725)	6 (4.35)	7 (5.07)	13 (9.42)
Flock 10	8 (5.80)	7 (5.07)	15 (10.87)	0 (0.00)	0 (0.00)	0 (0.00)	8 (5.80)	7 (5.07)	15 (10.87)
Flock 11	5 (3.625)	4 (2.90)	9 (6.52)	0 (0.00)	0 (0.00)	0 (0.00)	5 (3.625)	4 (2.90)	9 (6.52)
Flock 12	6 (4.35)	6 (4.35)	12 (8.70)	0 (0.00)	0 (0.00)	0 (0.00)	6 (4.35)	6 (4.35)	12 (8.70)
Total	68 (49.275)	70 (50.725)	138 (100)	1 (0.725)	2 (1.45)	3 (2.175)	67 (48.55)	68 (49.27)	135 (97.82)

[a] Number in the parentheses shows percentage of frequency

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[DOI: 10.21859/isv.4.1.29]

prevent the disease. Seropositive individuals should be segregated and culled and sheep should only be purchased from lentivirus-free flocks (20, 21). As an alternative to culling infected sheep, in order to reduce seroprevalence, it is suggested to segregate infected and non-infected sheep.

ELISA and AGID are the most frequently used techniques for diagnosis of Maedi-Visna virus infection. The results of many studies showed a consistent pattern of lower sensitivity values for the AGID than for the ELISA (22, 23). More-sophisticated alternatives for confirmation, such as immunoblotting and PCR techniques, did not find wide acceptance up to now because of complexity of the methods, resulting in difficult standardization (23). The ELISA currently used as screening test was shown to have the highest sensitivity and was thus confirmed as the best choice.

The present study provids an overview about the status of Maedi-Visna infection and its risk factors in sheep flocks in Kerman, Iran. To the best of our knowledge, this is the first serological survey of Maedi-Visna virus in sheep in Iran. Further investigations needs to be done to explore the seroconversion of Maedi-Visna virus in sheep and performance of the appropriate eradication program to prevent transmission through live animal trading. The results obtained in this study indicate that intensive sampling and testing combined with strict management may facilitate rapid eradication of Maedi-Visna virus from an infected flock.

This research was financially supported by the research council of Shahid Bahonar University of Kerman, Iran. The authors appreciate the assistance of Dr. Ali Asghar Mozaffari in sample collection.

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