

## Maedi-visna virus in Turkish sheep: a preliminary serological survey using ELISA tests

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**Abstract:** The maedi-visna virus (MVV) is distributed worldwide and, to date, no epidemiological surveys have been carried out using ELISA testing in Turkey. The aims of this study were as follows: i) to verify the diagnostic performance of a home-made ELISA test (HM-ELISA) using Turkish ovine sera in comparison with other commercially available ELISA tests, and ii) to perform a preliminary epidemiological survey in İstanbul province. The MVV seroprevalence in İstanbul province was 15.3%. Older sheep were 5 times more likely to be positive than younger animals, ewes were 3 times more likely to be positive than rams, and all the Red Karaman and White Karaman sheep tested were seronegative. The performance of the HM-ELISA and that of the other commercial tests using Turkish ovine serum samples was similar; therefore, the HM-ELISA test is suitable for use in extensive epidemiological surveys and eradication programs in Turkey.

**Key words:** ELISA, maedi-visna virus, sheep, Turkey, p25, gp44

### Türkiye'deki koyun ırklarında maedi-visna virüsü: ELISA testlerinin kullanılmasıyla yapılmış ön bir serolojik araştırma

**Özet:** Maedi-visna virüsü (MVV) dünyaca yaygın ve Türkiye'de de şu ana kadar yürütülmüş birkaç epidemiyolojik araştırmadan biridir. Bu çalışmanın amaçları 1) Türkiye'deki koyunların serumlarında ticari olarak mevcut diğer ELISA testleriyle karşılaştırılarak el yapımı ELISA testinin diyagnostik önemini kanıtlamak, 2) Türkiye'de İstanbul ilinde ön bir epidemiyolojik araştırma yapmaktır. İstanbul'da MVV seroprevalansı % 15,3 olarak saptanmış olup yaşlı koyunlar

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gençlerden 5 kat daha fazla hastalık oranına sahip bulunmuş, koyunlar koçlardan 3 defa daha fazla seropozitifliğe sahip bulunmuş, Morkaraman ve Akkaraman ırkları seronegatif bulunmuştur. Türkiye'deki koyun serumlarında, ticari olarak mevcut ve el yapımı ELISA'nın diyagnostik önemleri birbirine yakındır, bu yüzden el yapımı ELISA testi Türkiye'de ileride yapılacak daha geniş epidemiyolojik araştırmalar ve eradikasyon programları için uygun bulunmuştur.

**Anahtar sözcükler:** ELISA, maedi-visna virüsü, koyun, Türkiye, p25, gp44

Maedi-visna virus (MVV) is a lentivirus belonging to the family Retroviridae, is distributed worldwide, and causes the chronic multi-organ inflammatory disease maedi-visna (MV) in sheep. The infection is characterized by a long incubation period and slow progression, and clinical signs are usually observed many months or some years after infection. Non-suppurative encephalomyelitis (visna) and interstitial pneumonia (maedi) are the more frequently observed lesions in infected animals, although arthritis and lymphoproliferative mastitis with reduced milk production have also been described. The infection persists for life, despite the host's immune response (1). Infected animals are lifelong carriers, even in the absence of clinical symptoms, and can transmit the virus through the respiratory route, or via colostrum and milk (1,2). In the absence of an efficacious vaccine, the control of the infection is based on serological screening and slaughter of seropositive animals, and on buying uninfected sheep (3).

Serological tests are commonly used to detect infected animals. Several agar gel immunodiffusion tests (AGID) and enzyme-linked immunosorbent assay (ELISA) tests have been developed (4-7). The ELISA is the most suitable for screening a large number of serum samples. Due to high MVV antigenic variability, several ELISA tests have been evaluated in different geographic areas. In Turkey few epidemiological data on MVV are available (8).

In the present study a homemade MVV-ELISA test (HM-ELISA) was used to verify the seroprevalence of MVV in sheep in İstanbul province, Turkey. Two commercial methods (Pourquier and Bommeli) were used to validate the HM-ELISA using Turkish serum samples. Between June 2004 and December 2005, 542 ovine serum samples (129 males and 413 females) were randomly collected in İstanbul province. The sheep were from 4 flocks and the sampling included at

least the 50% of each flock's population (Table). The animals were aged between 6 months and 6 years, and were Kıvırcık, Red Karaman, Sakız, and White Karaman breeds. Each flock was composed of sheep of different breeds reared in a common environment under a semi-intensive system.

The 542 serum samples were tested using a MVV HM-ELISA (9). The test was based on the recombinant protein p25 and on the transmembrane subunit of the glycoprotein gp44. Production of the recombinant antigens and development of the ELISA test were conducted as previously described (10). Microwell plates were coated with synthetic peptide, serum samples were added, and after washing detection of the bound antibodies was performed with peroxidase-conjugate labeled protein G. Peroxidase activity was measured by adding a substrate chromogen solution. Color development was terminated after 30 min by the addition of sulfuric acid. Color intensity was measured at 450 nm in an ELISA reader (Bio Tek Instruments, Winooski, Vermont, USA), and the cut-off point was determined in each plate using a negative and a positive control sample (10). While a gold standard test is not available for MVV diagnosis, evaluation of the performance of the HM-ELISA test was based on comparison with 2 other reference tests. For this purpose, an additional 249 serum samples previously collected from sheep in different provinces of Turkey with a history of respiratory disease were tested using the HM-ELISA, and the 2 commercial ELISA tests (Pourquier [P-ELISA] and Bommeli [B-ELISA]), according to the manufacturer's instructions. The 249 serum samples were classified as positive if at least 2 of the 3 ELISA tests were positive, as negative if at least 2 tests were negative, and inconclusive if discordant results were obtained. To validate the homemade test statistical analysis was performed using the Epi Info<sup>TM</sup> freeware program from the CDC of Atlanta and Win Episcopo

Table. Serological results, estimated true prevalence of infection, and odds ratio calculated on the basis of the different breeds, age, and gender.

		Total sheep	Tested sera	Farm A	Farm B	Farm C	Farm D	Pos. sera	Pos. sera %	Neg. sera	Neg. sera %	Doubt. sera	Doubt. sera %	True preval.*	Odds ratio
Breed	Kıvırcık <sup>§</sup>	600	350	106	60	106	78	79	22.6	256	73.1	15	4.3	25.6	-
	Red Karaman	75	51	0	0	51	0	0	0	51	100	0	0	n.d.	-
	Sakız <sup>§</sup>	75	37	37	0	0	0	4	10.8	31	83.8	2	5.4	12.2	-
	White Karaman	200	104	0	52	0	52	0	0	104	100	0	0	n.d.	-
TOTAL		950	542	143	112	157	130	83	15.3	442	81.5	17	3.1	-	-
Age	< 1 year	-	250	93	37	71	49	28	11.2	212	84.8	10	4.0	-	1.0
	> 1 year	-	137	50	23	35	29	55	40.1	75	54.7	7	5.1	-	5.5
	TOTAL (age)*	-	387	143	60	106	78	83	21.4	287	74.2	17	4.4	-	-
Gen.	Female	-	140	52	22	39	27	22	15.7	112	79.4	6	4.3	-	3.3
	Male	-	110	41	15	32	22	6	5.4	100	90.9	4	3.6	-	1
	TOTAL (sex)**	-	250	93	37	71	49	28	11.2	212	84.8	10	4.0	-	-

(\*) The analysis was carried out considering only the breeds where at least one positive serum was found (§).

(\*\*) The analysis was carried out considering only the animals under 1 year old.

(11). Statistical analysis of the data included the kappa test and the Youden test. Once the sensitivity and specificity of each ELISA test were estimated, the Youden test was used to simultaneously evaluate the sensitivity and specificity of each ELISA (12). Subsequently, the kappa test was used to evaluate the concordance between the ELISA tests (13). Kappa values between 0.6 and 0.8 were considered to indicate good agreement between tests, and kappa values higher than 0.8 indicated very good agreement.

Once validated, the HM-ELISA test was used for a preliminary epidemiological survey of MVV infection in sheep in İstanbul province, according to breed, sex, and age. In particular, the samples were distributed into age groups (under or over 1 year of age). As the under 1-year-old group included mostly males (110 of 129), the sex variability was analyzed only in the animals in the over 1-year-old group in order to avoid distortion due to the samples. On the basis of the seronegativity

observed in Red Karaman and White Karaman sheep (Table), analysis of the seroprevalence in animals according to sex and age was performed only on the samples collected from Kıvırcık and Sakız sheep. The true prevalence values were obtained according to the values of sensitivity and specificity of the test (11).

The sensitivity and specificity values estimated for each test using Turkish ovine serum samples were, respectively, 97.08 and 90.37 for the Pourquier test, 77.27 and 99.23 for the Bommeli test, and 94.05 and 94.73 for the HM-ELISA test. Youden test results were 0.87, 0.76, and 0.88 for the Pourquier test, the Bommeli test, and the HM-ELISA test, respectively. The kappa values obtained based on 2-by-2 comparison of the tests were 0.733 for the P-ELISA vs. HM-ELISA, 0.657 for the B-ELISA vs. HM-ELISA, and 0.629 for the P-ELISA vs. B-ELISA. The HM-ELISA detected 83 of the 542 (15.3%) positive serum samples. Detailed results are reported in the Table.

To date, seroprevalence data obtained in Turkey using AGID tests have been inconsistent, such as 23.9% (14), from 3.8% to 41.2% (15), and 1.2% (8). These results have been obtained in different regions of Turkey and by different sampling methods; therefore, they are not properly comparable to the 15.3% prevalence observed in the present study. The HM-ELISA used for this epidemiological survey performed similarly as did the commercial Pourquier and Bommeli ELISA tests, as kappa values higher than 0.6 were obtained by the 2-by-2 comparison. Based on our experience, the HM-ELISA test is less expensive than the other ELISA tests and is more suitable for extensive epidemiological studies in Turkish flocks.

The preliminary results obtained in the present study confirm, as expected, that older sheep were 5 times more likely to be positive than younger sheep (O.R.: 5.5; I.C.: 3.18%-9.73; 95% L.C; chi square: 45.38;  $P < 0.0001$ ) (Table). Considering the odds ratio value as relative risk, females were 3 times more likely to be positive than males (O.R.: 3.27; I.C.: 1.2%-9.43; 95% L.C; chi square: 5.64;  $P < 0.01$ ) (Table). Higher seroprevalence in ewes than in rams has been observed in previous epidemiological studies, which was attributed to differences in management practices between males and females (16). In contrast to ewes, rams are often kept in small groups, and limited contact between animals limits MVV transmission.

The present study's most relevant results were those on seroprevalence according to breed. All Red Karaman and White Karaman test results were negative for MVV, while Kıvırcık sheep had the highest seroprevalence. As described in the Table, composition of the flocks was mixed, and Red Karaman and White Karaman sheep were reared with other ovine breeds that were MVV positive. There was no separation of animals within the flocks and sheep

of different breeds were in direct contact with each other. At each of the 4 farms the semi-intensive rearing conditions were identical. Previous studies reported that some ovine breeds are more susceptible to MVV infection than others (17), but the present study is the first to suggest differences in susceptibility in Turkish sheep according to breed.

In Turkey no eradication or control programs for MVV exist. There are about 25 million sheep in Turkey, of which about 48,000 are reared in İstanbul province. Red Karaman and White Karaman sheep are the most common breeds in Turkey. The finding that these breeds seem to be resistant to MVV infection is encouraging. In contrast, Kıvırcık sheep seem to be the most susceptible to MVV infection. These sheep are reared in a limited number in Turkey, but are the most common breed in İstanbul province. These sheep have historical value and produce a traditional cheese; therefore, their replacement with MVV-resistant breeds should not be encouraged. Nonetheless, more extensive epidemiological studies are necessary to confirm the differences in susceptibility according to breed observed in the present study.

Due to economic factors, obtaining uninfected flocks is an important goal of Turkish farmers. The most reliable way to reduce MVV prevalence in Turkish flocks is early detection of infected animals, isolating them until slaughter, and replacing them with uninfected sheep. Considering the importance of MVV transmission via colostrum, all pregnant ewes should be tested, and if positive their lambs should be artificially reared (18). The HM-ELISA test used in the present study could be used for these typing purposes, as it is less expensive than the other methods and as efficacious as other tests using Turkish ovine serum samples.

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