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Investigation of the Seroprevalance of Maedi-Visna in the Region of Van Using Elisa and Histopathological Findings

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Abstract: In the present study, seroprevalance of maedi-visna in sheep in the region of Van was aimed to investigate. A total of 465 serum samples obtained from sheep raised in Van and its towns were investigated with regard to maedi-visna specific antibody using ELISA procedure. Out of 465 samples 30 of them were seropositive (6.45%) and 20 of them were doubtful (4.30%) according to test procedure. Histopathology was also applied to some of these sheep which slaughtered in an abattoir after blood sampling. According to histopathological analysis 9 doubtful samples were also found to be positive. But, due to mild lymphoproliferation in there samples ELISA gave doubtful results. As a result with this study, seroprevalance of maedi-visna was determined in the region of Van and in the doubtful cases histopathological assessments believed to strengthen serological findings.

Key words: Maedi-visna, sheep, ELISA, serology, seroprevalance, histopathology

INTRODUCTION

Maedi-visna is caused by the non-onchogenic Maedi-Visna Virus (VMV) which is the Retroviridae family belonging to lentivirus subfamily. Infections of lentivirus are characterized by a very long incubation period and a slow and progressive development of disease (Dawson, 1980; Georgsson and Pálsson, 1971; Jones *et al*., 1997; Narayan and Clements, 1989; Pépin *et al*., 1998). Filogenetic relation between maedi-visna virus and Caprine Arthritis Encephalitis Virus (CAEV) has been reported (Ravazzolo *et al*., 2001). Pulmoner form of the diseased is called Ovine Progressive Pneumonia (OPP) and characterized with interstitial pneumonia (Georgsson and Pálsson, 1971). On the other hand, visna form of the disease is characterized with central nervous system symptoms such as meningoencephalitis. Recent reports related the disease demonstrated that neither of these sites had previously been regarded as a target for MVV infection, in the cytoplasm of epithelial cells of the third eyelid, heart, liver and kidneys of naturally infected sheep (Angelopoulou *et al*., 2006; Brellou *et al*., 2007; Capucchio *et al*., 2003). The disease has been reported from all over the world and cause important economical losses (Angelopoulou *et al*., 2005).

Infection is occur as a result of ingestion of infected mononuclear cell containing colostrums by lambs and keeping healthy sheep and together with sheep having pulmoner adenomatosis in crowded environment play important role in spread of the disease (Aslantas *et al*., 2002; Imren and Sahal, 1991). Furthermore, pulmonary aerosols semen has also been reported to have role in the transmitted of the disease (Capucchio *et al*., 2003).

In the maedi form of the disease; precise respiration difficulty, increase in body temperature, body weight loss although they have normal appetite are the important clinical signs of the disease. In the visna form of the disease; trembling the lips, inclining in the head and ears, being in the end of flock, paresis and paralysis starting from lind limb to forefoot are the signs of the disease (Imren and Sahal, 1991).

In the necroscopic pulmoner form; macroscopic lesions characteristic to the disease are seen. When thorax are opened; lungs are not deflated and sometimes costal prints on the lungs are prevent. Lungs become bigger than normal and get 2-5 times heavier. In the histopathological examination; in the early stage; interalveoler capillaries are hyperemic, alveolar septa are thickened as a result of lymphocytes and macrophages infiltration and also smooth muscle hyperplasia in alveolar

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ducts and in terminal bronchioles, lymhofollicular proliferation in perivascular, peribronchioles and bronchioles have been reported (Dungworth, 1993; Jones *et al*., 1997). Definitive diagnosis of the disease has been made together with clinical symptoms, serological tests (agar gel immunodiffusion, complement fixation, indirect immunofluorescence and ELISA) confirmated by macroscopical and histopathologic findings (Fournier *et al*., 2006; Imren and Sahal, 1991).

In our country, the disease is first reported by Alibasoglu and Arda in 1978 in a sheep slaughtered in an abattoir. Later on a few more studies had also been made by different researchers (Aslantas *et al*., 2002; Burgu *et al*., 1990; Yilmaz *et al*., 2002).

In the present study, seroprevalance of maedi-visna infection in sheep raised in the region of Van were aimed to investigate using ELISA and histopathological examination.

MATERIALS AND METHODS

In the present study a total of 465 sheep were used which were obtained from the city of Van and it's provinces (Gurpinar, Gevas, Catak, Muradiye and Caldiran).

The sheep were aged between 2-5 years old from both sexes. For this purpose; 10 mL of blood were taken from jugular vein to obtained serum samples were kept at -20°C until use.

Out of these 465 sheep, 110 of them were about to slaughter in an abattoir. Therefore, blood samplings were made just before they were slaughtered. Thus, lung samples were also collected from these 110 slaughtered sheep to examine histopathologically.

ELISA test: Frozen serum samples were left until they became to room temperature. They were then examined for the presence of antibodies, developed against maedi-visna virus using a commercial test kit (Maedi-Visna/CAEV serodiagnosis by ELISA test in serum, Pourquier ELISA Maedi-visna/CAEV serum screening version, P00303/06, Institute Pourquier, France).

The protocols were applied to the serum samples as mentioned in the test procedure and OD values were obtained at 450 nm. As reported in the test protocol; S/P ratio under 110% considered negative, between 110-120% considered doubtful and over 120% considered as positive.

Histopathological examination: The lung samples (n = 110) were fixed in 10% buffered formaline and paraffin-embedded, sectioned at 4-6 μm processed according to routine procedures and stained with Hematoxylen and Eosin (HE) for histopathologically examination. Typical maedi-visna histopathology was evaluated in the lungs for the presence of: Lymphofollicular proliferations, thickening of alveolar walls and smooth muscle hyperplasia. These findings were graded as mild, moderate and severe as demonstrated in Table 1 (Fournier *et al*., 2006).

RESULTS

In the present study, the sheep had no clinical symptoms of maedi-visna such as weight loss respiratory system disorder, arthritis or Central Nervous System (CNS) disorders. A hundred and ten sheep out of 465 were slaughtered. Therefore, in the macroscopic examination of these lungs; infected lungs were bigger and heavier. These lungs were not collapsed, had pale color, were in rubber consistency and impression of ribs on the lungs were apparent.

Out of 465 serum samples, 30 (6.45%) were found to be positive, 20 (4.30%) were doubtful and 415 (89.25%) were negative serologically using ELISA. Out of 110 histopathologically examined sheep, 13 (11.81%) were positive and 9 (8.18%) were doubtful in ELISA. In the serologically positive samples, 3 typical histopathological findings of maedi were graded moderate (Table 1). The first finding was lymphofollicullar proliferations that often had germinal centers seen around the vessels, bronches and bronchioles (Fig. 1). The second finding was the thickening of the alveolar septa through lymphocyte and macrophage infiltration. The third finding was smooth muscle hyperplasia in the alveolar ducts and terminal bronchioles walls (Fig. 2). In the histopathological examinations of doubtful samples determined by ELISA; smooth muscle hyperplasia and thickening in the alveolar septa was moderate and these lesions were similar to positive samples. But lymphofollicullar proliferation were mild (Table 1).

Table 1: Grading system used for assessing the severity of histopathologic lesions typical of Maedi-Visna (MV) virus infection in the lung (Fournier *et al*., 2006)

Smooth muscle hyperplasi	Lymphofollicular proliferations	Diffuse thickening of alveolar septa
Mild	Rare lymphoid follicles with absence of germinal centers	Few lymphocytes and macrophages in alveolar septa
Moderate	Small to moderate numbers of lymphoid follicles often with presence of germinal centers	Moderate numbers of lymphocytes and macrophages in alveolar septa
Severe	Numerous lymphoid follicles predominantly with germinal centers	Numerous lymphocytes and macrophages in alveolar septa

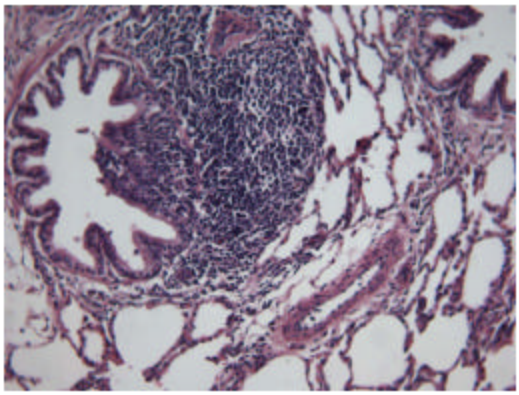

Fig. 1: Chronic progressive pneumonia (maedi). Lymphofollicular proliferations. HE, ×520

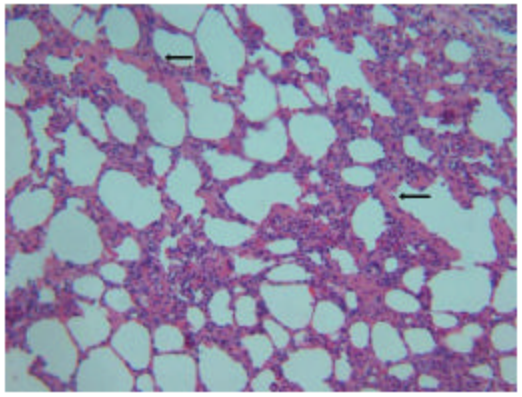

Fig. 2: Chronic progressive pneumonia (maedi). Thickening of alveolar septa and smooth muscle hyperplasia HE, ×520

DISCUSSION

Apart from Australia and New Zealand maedi-visna has been reported from different parts of the world. The disease reported to cause important economical loses (Dawson, 1980; Honger *et al*., 1990; Kita *et al*., 1990; Schaller *et al*., 2000).

Seroprevelance studies have been performed for several diseases to determine prevalence of diseases in many regions. Obtained results expected to help in taking precautions. Especially in the regions, which respiratory system diseases are spread in sheep, determining etiological factors will help in combating diseases and is important in guessing prognosis.

In the present study, although the sheep used in the present study had no clinical symptoms of the disease, the seropositivity to maedi-visna was 6.45%. Therefore, routinely made serological studies especially with concert to such diseases are important and for this respect this study is the first investigation in this region, thus, should be taken seriously by the animal procedures and government bodies.

Studies with concern to seroprevalance of maedi-visna; Schaller *et al*. (2000) found 9% in Switzerland, Honger *et al*. (1990) 9.5% in Austria, Simard and Morley (1991) 19% in Canada, Kita *et al*. (1990) 24% in Poland, Sihvonen *et al*. (1999) 1.6% in Finland, Boulujihad and Leipold (1994) 24.8% in Morocco, Cutlip *et al*. (1992) 26% in USA and Giangaspero *et al*. (1993) found 6% in Syria with regard to seropositivity to maedi-visna.

Studies with regard to seroprevalance of maedi-visna in our country, Burgu *et al*. (1990) found 23.9% seropositivity out of 12 sheep flocks, Cimtay *et al*. (2004) found 10% in Sanliurfa, a serological and histopathological study were performed by Yilmaz *et al*. (2002) in Istanbul and found 1.2% seropositivity to maedi-visna. In other studies performed in our country the seropositivity were also found differently at different regions as 1.5, 2.29 and 2.6% (Aslantas *et al*., 2002). In the present study 30 samples (6.45%) were found to be seropositive with regard to maedi-visna.

In the histopathology, out of investigated 110 lung samples, 13 (11.81%) of them positive and 9 (8.18%) were doubtful in ELISA. In those 13 positive samples determined by ELISA, all of them showed all typical histopathological findings of maedi-visna. In those 9 doubtful samples determined by ELISA; In those 13 positive samples determined by ELISA, all of them showed all typical histopathological findings of maedi. In those 9 doubtful samples determined by ELISA, the thickening of the alveolar septa and smooth muscle hyperplasia was moderate. But lymphoproliferation was mild. Therefore, mild lymphoproliferation and thus mild infection most probably caused doubtful result in ELISA.

CONCLUSION

As a result the seroprevalence of the maedi-visna were determined first time to be 6.45% and in doubtful cases; histopathology could be helpful in the definitive diagnosis of the disease.

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REFERENCES

Angelopoulou, K., G.D. Brellou and I. Vlemmas, 2006. Detection of maedi-visna virus in the kidneys of naturally infected sheep. J. Comp. Path., 134: 329-335. PMID: 16709422.

Angelopoulou, K., K. Karanikolaou, M. Papanastasopoulou, M. Koumpati-Artopiou, I. Vlemmas, O. Papadopoulos and G. Koptopoulos, 2005. First partial characterisation of small ruminant lentiviruses from Greece. Vet. Microbiol., 10, 109 (1-2): 1-9. DOI: 10.1016/j.vetmic.2005.04.025. PMID: 15964721.

Aslantas, O., D. Pinar and B. Gungor, 2002. Serologic Investigation of Maedi-Visna Infection in Hatay Region. Vet. Hek. Mikrobiyol. Derg., 2: 31-34. http://www.mikrobiyoloji.org/pdfler/703020207.pdf.

Boulujihad, M. and H.W. Leipold, 1994. Ovine lentiviral infection (maedi-visna) in Morocco: A serologic and postmortem survey. Zetralbl. Veterinarmed. A., 41: 317-328. PMID: 7975974.

Brellou, G.D., K. Angelopoulou, T. Poutahidis and I. Vlemmas, 2007. Detection of Maedi-Visna Virus in the Liver and Heart of Naturally Infected Sheep. J. Comp. Path., 136: 27-35. DOI: 10.1016/j.jcpa.2006.10.001. PMID: 17258227.

Burgu, I., A. Toker, Y. Akca, F. Aklan, Z. Yazici and A. Ozkul, 1990. Serological investigations on visna-maedi virus infection of sheep in Turkey. A. Ü. Vet. Fak. Derg., 37: 538-553. http://dergiler.ankara.edu.tr/dergiler/11/596/7622.pdf.

Capucchio, M.T., E. Sanna, M.P. Sanna, S. Farigu, R. Mineli and F. Guarda, 2003. Maedi-visna virus detection in ovine third eyelids. J. Comp. Path., 129: 37-43. PMID: 12859906.

Cimtay, I., O. Keskin and T. Sahin, 2004. Investigation on some lentivirus infections in sheep and goats in region of Sanliurfa. Uludag Univ. J. Fac. Vet. Med., 23: 33-38. http://kutuphane.uludag.edu.tr/PDF/vet/2004-23(1-2-3)/mak06.pdf.

Cutlip, R.C., H.D. Lehmkuhl, J.M. Sacks and A.L. Weaver, 1992. Seroprevalance of ovine progressive pneumonia virus in sheep in the United States as assessed by analyses of voluntarily submitted samples. Am. J. Vet. Res., 53: 976-979. PMID: 1320816.

Dawson, M., 1980. Maedi-visna: A review. Vet. Rec., 106: 212-216. PMID: 6244690.

Dungworth, D.L., 1993. The Respiratory System. 4th Edn. In: Jubb, K.V.F., P.C. Kennedy and N. Palmer (Eds.). Pathology of Domestic Animals, Academic Press Inc., San Diego, USA, 2: 629-631. ISBN: 0123916070.

Fournier, D., J.R. Campbell and D.M. Middleton, 2006. Prevalence of maedi-visna infection in culled ewes in Alberta. Can. Vet. J., 47: 460-466. PMID: 16734372.

Georgsson, G. and P. Pálsson, 1971. The histopathology of maedi: A slow viral pneumonia of sheep. Vet. Pathol., 8: 63-80. PMID: 5157932.

Giangaspero, M., D. Tabbaa, H. Nishikawa and E. Vanopdenbosh, 1993. Epidemiological survey of the Maedi Visna (MV) virus in Syrian Awassi sheep. Rev. Elev. Med. Vet. Pays. Trop., 46: 431-434. PMID: 8190977.

Honger, D., B. Leitold and W. Schuller, 1990. Serological studies of antibodies against maedi-visna virus in sheep in Austria. Berl Munch Tierarztl Wochenschr, 103: 39-41. PMID: 2154964.

Imren, H.Y. and M. Sahal, 1991. Veterinary Internal Diseases. Feryal Press. 2nd Edn. Ankara, pp: 230-231. ISBN: 975-7774-00-6.

Jones, T.C., R.D. Hunt and N.W. King, 1997. Diseases Caused by Viruses. 6th Edn. Veterinary Pathology. In: T.C. Jones, R.D. Hunt and N.W. King (Eds.). Williams and Wilkins, Baltimore, pp: 197-370. ISBN: 068304481-8.

Kita, J., R.C. Cutlip, W. Kenpski and J. Sachs, 1990. Survey of antibodies against maedi-visna in sheep in Polands. Pol. Arch. Weter, 30: 5-11. PMID: 1966747.

Narayan, O. and J.E. Clements, 1989. Biology and pathogenesis of lentiviruses. J. Gen. Virol., 70: 1617-1639. PMID: 2544657.

Pépin, M., C. Vitu, P. Russo, J.F. Mornex and E. Peterhans, 1998. Maedi-visna virus infection in sheep: A review. Vet. Res., 9: 341-367. http://www.vetres-archive.org//file/Vet.Res._0928-4249_1998_29_3-4_ART0008.pdf.

Ravazzolo, A.P., D. Reischak, E. Peterhans and R. Zanoni, 2001. Phylogenetic analysis of small lentiviruses from Southern Brazil. Virus Res., 79: 117-123. DOI: 10.1016/S0168-1702(01)00339-2.

Schaller, P., H.R. Vogt, M. Strasser, P.F. Nettleton, E. Peterhans and R. Zanoni, 2000. Seroprevalance of maedi-visna virus and border disease in Switzerland. Schweiz. Arch. Tierheilk., 142: 145-153. http://www.unboundmedicine.com/medline/ebm/record/10804839/abstract/[Seroprevalence_of_maedi_visna_and_border_disease_in_Switzerland].

Sihvonen, L., V. Hirvela-Koski, L. Nuotio and M. Kokkonen, 1999. Serological survey and epidemiological investigation of maedi-visna in sheep in Finland. Vet. Microbiol., 65: 265-270. DOI: 10.1016/S0378-1135(98)00312-5.

Simard, C. and R. Morley, 1991. Seroprevalance of maedi-visna in Canadian sheep. Can. J. Vet. Res., 55: 269-273. http://www.pubmedcentral.nih.gov/picrender.fcgi?artid=1263463&blobtype=pdf.

Yilmaz, H., A. Gurel, N. Turan, T. Bilal, B. Kuscu, M.M. Dawson and K.L. Morgan, 2002. Abattoir study of maedi-visna virus infection in Turkey. Vet. Rec., 21: 358-60. PMID: 12371695.