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Full Length Research Paper

Haematological parameters in blood of maedi-visna virus-infected and uninfected sheep

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The maedi-visna virus (MVV) classified as a lentivirus of the retroviridae family, causes a very common economically important disease in sheep, in many parts of the worlds. Presences of the infection in Turkey have been shown by researches in previous studies. In this study all blood samples were examined by ELISA and PCR to detect MVV antibody and antigen responses, respectively. Hematological findings were monitored and comparing antibody and antigen positive naturally infected (n= 5), antibody positive and antigen negative (n=20), antibody and antigen negative (n=20) in sheep was done. When infected sheep were compared with control sheep, Hgb and MPV parameters were shown statistically different (P 0.001). These findings suggested that MVV infections should be considered as an important health risk for sheep flock and Hgb and MPV blood parameters may be helpful to diagnosis of MVV.

Key words: ELISA, hematology, lentivirus, PCR, sheep.

INTRODUCTION

Maedi- visna virus (MVV) is a lentivirus in retroviridae family, currently referred to as small ruminant lentivirus (SRLVs) that causes slow progressive multi-systemic diseases in sheep (Capucchio et al., 2003; Angelopoulou et a., 2006). The main pathological manifestations are interstitial pneumonia (Maedi), encephalomyelitis (Visna), mastitis and, arthritis (Straub, 2004; Benavides et al., 2009; Carrozza et al., 2010). Visna occurs much less than maedi in sheep flocks (Reina et al., 2010). Transmission can be occurred via aerosol with close contact and colostrum, milk, semen and, intrauterine (Straub, 2004; Reina et al., 2010). The main targets of SRLVs are the lungs, brain, mammary glands, joints, lymph nodes, spleen and bone marrow. MVV was also detected in the third eyelid of sheep. They suggested that the third eyelid may play a role in disease transmission. In some studies, no differences in milk production have been demonstrated between seropositive and seronegative dairy ewes. Vertical transmission of SRLVs has been reported only occasionally and the use of semen from seropositive rams does not seem to be a major risk factor in sheep (Reina et al., 2009). Lentivirus diseases are commonly seen worldwide (Benavides et al., 2009). Caprine arthritis encephalitis virus (CAEV) is closely related to MVV (Cortez-Romero et al., 2010). MVV was the first lentivirus to be isolated and characterized in Iceland in 1957 (Sigurdsson et al., 1953). MVV specific antibodies were found in the different countries such as Greece, Spain, Morocco and Turkey (Seimenis et al., 1982; Gonzalez et al., 1983; Mahin et al., 1984; Yilmaz et al., 2002; De Andrésa et al., 2005; Preziuso et al., 2010). Some affected animals emaciate without showing clinical signs until their death. Following infection, animals produce anti-viral antibodies in a few weeks to several months time. The main cells infected *in vivo* are monocytes and macrophages, with the bone marrow serving as a reservoir of infection. Virus is disseminated to the target tissues via the blood stream inside monocytes (De Andrésa et al., 2005). The somatic cell counts are increased in infected sheep. Death can be occurred by MVV syndromes in two years. Hypochromic

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anemia, leucocytosis, lymphocytosis, hyper-gamma globulinemia are often seen in infected sheep with MVV (Lipecka et al., 2010; Reina et al., 2010). Serological tests including agar gel immunodiffusion (AGID), enzyme-linked immuno sorbent assay (ELISA), western blotting (WB), radio immuno precipitation (RIPA) and radio immuno assay (RIA) are common used to detect antigen or specific antibody to MVV (Yilmaz et al., 2002; Lipecka et al., 2010; Reina et al., 2010) . ELISA and AGID are preferred used for detecting MVV infected animals. PCR has also been used for detection of virus in blood and other tissues. There are two main difficulties in developing suitable PCR tests for SRLV heterogeneity and low virus load *in vivo* (De Andrésa et al., 2005; Reina et al., 2010). In despite of easily diagnosis of MVV a vaccine is not being used (Belknap, 2002).

There are many studies about serologically diagnosis of MVV however the comprehensive data hematologically was limited (De Andrésa et al., 2005; Preziuso et al., 2010). This study is aimed to compare to some hematological variables in naturally infected sheep with MVV from uninfected animals.

MATERIALS AND METHODS

Study area and sample collection

The present study was conducted on two hundred and fifty sheep during the period from July to December 2010 at the Kirikkale, Turkey. Kirikkale is situated in central Anatolia region of Turkey in Asia. It lays 39º 52' 53" north of the equator and 33º 26’ 46” meridians east of Greenwich. Kirikkale has a coastline with the majority lying along the Kizilirmak River. Kirikkale's climate is a mixture of wet and dry. The northern part of the region is hilly and in many places the altitude ranges between 700 and 1450 m (2296-4757 ft) above sea level. Extreme recorded temperatures range from -10°C (14°F) to 35°C (95°F) at Kirikkale. Samples were collected from all sheep within herds under investigation at the same time of clinical examination. 10 ml blood sample from each animal was collected from jugular vein using vacutainer tubes. Samples were allowed to clot and transferred onto ice as quickly as possible to the laboratory of the Kirikkale University, Faculty of Veterinary. The sera were separated by centrifugation at 2000 rpm for 10 min and aspirated in Eppendorf tubes and stored at -20°C until testing.

Clinical examination

Sheep were clinically examined by the researchers for the presence of clinical signs, which could suggest the infection. Animals used in this study did not show any apparent clinical signs of MVV infection.

Enzyme-linked immuno sorbent assay (ELISA)

Sera and whole blood samples were collected from sheep in Kirikkale province. A commercial ELISA kit (Pourquier ELISA Maedi-Visna/CAEV serum Screening /France) was used for the detection of antibody response according to the manufacturer's instructions. Samples were analyzed and calculated with an automated ELISA reader at 450 nm (SIRIO S® Elisa Reader, Indonesia) (Azkur et al., 2011).

Nested polymerase chain reaction (PCR)

DNA was extracted directly from whole blood DNA Isolation Kit (QIAGEN® GmbH, Germany) according to the manufacturer's instructions. DNA was stored at - 20°C until PCR was performed. After DNA isolation, the first round PCR was run in reaction mixture contained 5 ul cDNA, 25 mM Tris-HCl pH 8.9, 50 mM KC1, 3 mM $MgCl_2$, 0.2 mM dithiothreitol, 2 mM of dNTP mix, 10 pmol of each external primer "LenUpl" and "LenDo2" and 3 U Taq DNA polymerase (MBI Fermentas)[17]. Ten microliters nested PCR reactions were performed in a reaction mixture with the same reagent composition using the internal primers "LenNestUp3" and "LenNestDo4"[17] (Eltahir et al., 2006) . All nested-PCR reactions were carried out in BO-PCR-5 thermal cycler (Hamburg, Germany). The InGenius LHR (Syngene, Cambridge, UK) was used to see PCR amplicons by using ethidium bromide staining after 1.5% agarose gel electrophoresis (Azkur et al., 2011; Sambrook et al., 1989)

Haematological analysis

Collected blood samples were analyzed with hematological analyzer (MS-9, France) for white blood cell count (WBC), red blood cell (RBC), mean corpuscular volume (MCV), mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, hemoglobin, packed cell volume, and platelet.

Statistical analysis

Data analyses were carried out using statistical software program (SPSS for Windows, Version 15.0, SPSS Inc., Chicago, USA). Association between the occurrence of MVV infection and the hypothesized risk factors was differences between the groups of sheep were analyzed by Kruskal-Wallis test and Mann-Whitney test; p value of less than 0.05 was considered significant (Ergun and Aktas, 2009).

RESULTS

To find sheep infected with MVV, antibody response was first investigated by using ELISA in sera. It was found twenty five sheep have shown antibody response and twenty sheep have not shown any antibody response against to MVV. All samples were examined to detect MVV antigen by using nested PCR. It was found that five sheep have shown antigen signal for specific MVV. MVV was found by using nested PCR and previously described primer pairs LenUpl1, LenDo2 and LenNestUp3 and LenNestDo4 in samples. When RBC patterns were examined significant, differences were found between groups 1 and 3 (Table 1). WBC, MCV and MCHC values were significantly higher in the group 1 than group 2 and 3 (P 0.05) . Hgb, MPV values in group 1 are significantly higher than other groups (P 0.05). RBC, MCH, Hgb values in group 1 was statistically different from group 3 (P 0.05). There were significant differences in MPV values between groups 2 and 3 (P 0.001). The platelet counts were higher in group 2 than groups 1 and

Table 1. Mean ± SD of the haematological values for group 1 (n=5), 2 (n=20) and 3 (n=20).

Parameters	Lentivirus ab+/ ag + (Group 1)	Lentivirus ab-/ ag – (Group 2)	Lentivirus ab+/ ag – (Group 3)
WBC(n/10³)	69.6±25.7	67.26±1834	62.91±19.43
Lym%	82.94±4.60	79.41±5.64	77.94±5.46
Mon%	0.440±0.261	0.4100±0.2864	0.4350±0.1424
NGr%	16.62±4.66	20.17±5.65	21.64±5.47
Lym#	58.4±22.9	53.74±15.88	49.68±17.56
Mon#	0.340±0.288	0.3150±0.3183	0.2900±0.1744
NGr#	10.90±3.13	13.200±3.862	12.950±3.151
RBC(n/10^9)	9.802±1.107	9.201±1.140	9.010±1.164
MCV(fl)	34.20±2.98	33.525±3.184	33.920±2.970
Hct(%)	33.52±4.64	30.745±4.077	30.190±4.357
MCH (pg)	11.100±0.711	10.935±1.570	10.935±0.625
MCHC (g/dl)	32.572±1.055	32.675±4.142	32.595±2.082
Hgb(g/dl)	10.880±1.303	9.935±1.609	9.820±1.371
Plt(n/mm³)	397.6±145.8	619.1±192.7	430.5±187.3
MPV (fl)	5.940±1.254	5.255±0.808	5.635±0.921

3. Additionally platelet counts were statistically different between three groups (P 0.05). Elevated Lym% and decreased N/Gr% counts are showed statistically different compare with between groups 1 and 2 or groups 1 and 3 (P 0.05). Mon% were higher in group 1 than groups 2 and 3 (P 0.05). Hct values are higher in group 1 than groups 2 and 3 but the differences are not significant statistically (P 0.05).

DISCUSSION

In previous work we have shown different patterns in hematological parameters in blood of infected MVV (group 1) and positive antibody, negative antigen against MVV (group 3) compared with the uninfected sheep (group 2). Serological tests are commonly used to detect infected animals. De Andrésa et al., (2005) reports that ELISA is generally a more sensitive technique than AGID test for the detection of SRLV antibodies and supple-mentary tests such as RIA, RIPA, and WB are useful assays but not enough as true gold standard methods for SRLV. Additionally PCR tests are generally less sensitive than ELISA techniques due to the low viral load in the post seroconversion phase of infection (De Andrésa et al., 2005). In the present study a MVV-ELISA test (Pourquier ELISA) was used to detect MVV in sheep in Kirikkale province, Turkey. Sero epidemiological studies of MVV in sheep have been reported in many countries including Turkey such as 3% up to 42%. (Campbell et al., 1994; Angelopoulou et al., 2006; Eltahir et al., 2006; Lipecka et al., 2010; Yilmaz et al., 2002). In previous studies reported that whether interstitial pneumonia, encephalomyelitis, mastitis, and arthritis can be seen or can show any clinical signs with infected MVV in sheep (Straub, 2004; Benavides et al., 2009; Carrozza et al., 2010). In this study we didn't determine any clinical signs such as; lameness, weight loss, coughing, fever, ataxia, abnormal milk in relation to MVV infection in the sheep flock. The impact of MVV infection on the hematological parameter of peripheral blood is unknown. WBC patterns changed between groups due to changes in immunity as a consequence of viral infection. This result can be supported by Lipecka et al. (2010). RBC, MCH, Hgb values in group 1 was statistically different from group 3 (Table 1). Ballesteros et al. (2010) reported that an increase in the Hematocrit and RBC value and Hgb concentration significantly different but they were not detected by any statistically significant differences in MCV, MCH, MCHC values between MVV positive and negative animals. RBC, MCH and Hgb has notspecific parameters for MVV or any diseases; therefore changes of these parameters could be associated with other conditions (Gazyagci et al., 2010). Lipecka et al. (2010) reported that monocytes, macrophages lineage and mononuclear cells are the target for MVV (Lipecka et al., 2010). Ballesteros et al. (2010) founded that lymphocytes and monocytes in percentage were not significantly different between positive and negative animals. We also found higher Mon and lymphocyte in percentage in infected group 1 than other groups (Table 1). We are in agreement with the previous reports about mechanism regarding production of some cytokines, mainly interferon. These findings suggest that MMV infections should be considered a health risk for sheep flock and may have a role in the changes Hgb and MPV made in the host and the study provide very interesting data which may aid diagnosis with a predictive value, as well as provide

information to better understand the pathogenesis of the infection.

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