



The changes in the levels of elements in sheep with Contagious Ecthyma

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ABSTRACT

This study has aimed to investigate the differences between the levels of trace elements in sheep with the diagnosis of ecthyma, and in healthy sheep. For this purpose, Be, Bi, Pb, Cd, Na, K, Ca, Mg, Fe, Cu, Zn, Se, Ni and Mn, Co, Cr, Li elements in the plasma of sheep with Contagious ecthyma detected with PCR and in sheep in the control group were analyzed with ICP-OES. In this study, the levels of Fe, Cu, Zn, Se and Pb trace elements were found to be statistically different between the sheep infected with parapoxvirus. The Na element of the macro elements was observed to be statistically different in sheep with ecthyma, to the control group ($P < 0.05$). When there are low levels of metal concentration in sheep with ecthyma, their immune system can be considered to be weakened. Further studies can be carried out on the increase in the risk of developing the disease as a result of, in particular, a decrease in the Fe, Cu, Zn and Se concentrations in sheep infected with the virus.

Key words: Contagious ecthyma, ICP-OES, Makro-Trace elements, PCR, Sheep.

INTRODUCTION

The agent for Contagious Ecthyma (Orf)(CE), which is widely seen in sheep and goats, is in the parapoxvirus genus of the poxviridae family, and it is a DNA virus. Generally, it manifests with vesicles and pustules in areas of the body without wool, such as the mouth and nose of young animals. The rate of morbidity of the infection is 80%, whereas in young animals, due to malnutrition, immunosuppression and secondary infections, it may result in a mortality of 5-10% (Nandi and Chowdhury, 2011). This zoonotic disease may be transmitted to humans through contact with the infected animal, and it may cause lesions on the hands, fingers, and rarely on the face (Karakas *et al.*, 2013; Oguzoglu *et al.*, 2014). The CE infection is common worldwide, and it is also frequently seen in our country (Ince *et al.*, 2016; Yoldar *et al.*, 2016).

Macro and trace elements, which are present in all living organisms, and required for the biological functions, are carried to the organisms by environmental convection, through air, soil, food and drinking water (Saleem *et al.*, 2016; Krzysztof *et al.*, 2016). When the minerals are absent, the immune system is suppressed, leading to an increased risk of infection (Scrimshaw, 2003). Beside all these factors, the deficiencies of minerals that enable the maintenance of immune functions, and the other macro and micro nutrients, increase the susceptibility to infections (Nieman, 1994). In this study, it was aimed to investigate the differences between the levels of trace elements in sheep with the diagnosis of CE and in healthy sheep.

MATERIALS AND METHODS

Blood samples were collected from 20 sheep that showed signs of parapoxvirus infection from a herd located in the Alakoy town of Tusba, a district of the Van province, and from 30 healthy sheep (control group) that showed no infection signs as the control group.

Virological Examination

DNA extraction and seminested PCR. Obtained blood samples in EDTA tubes were centrifuged at 2000 rpm for 10 min. and leukocyte separated into the stock tubes, and stored at -8°C until to the test. Viral DNA was extracted from 200 µl volumes of each sample using by High Pure Viral Nucleic Acid Extraction kit (Roche, Germany) according to manufacturer instructions. A size of 594 bp for B2L gene of parapox virus was amplified by seminested PCR, using primer set as described by Inoshima *et al.* (2002).

The primers PPP1 (5' gtc gtc cac gat gag cag ct-3') and PPP3 (5'- gcg agt ccg aga aga ata cg-3') were used for the first amplification, while primers PPP1 (5' gtc gtc cac gat gag cag ct-3') and PPP4 (5'-tac gtg gga agc gcc tcg ct-3') were used for the second amplification under same situations in thermal cycler. The PCR mix, with a total volume of 30 µl, contained 3 µl of extracted DNA, 3 µl of Taq buffer (10×, 750 mM Tris-HCl, 200 mM (NH₄)₂SO₄, 0.1% (v/v) Tween 20), 2 µl of the MgCl₂ (25mM), 1 µl of each primer (10mM), 1 µl of the four deoxynucleoside triphosphates (10 mM) solution, 0,25 µl of Taq DNA polymerase (5U/µl), and 18,75 µl of molecular biology grade water (Thermoscientific, USA). Sterile molecular biology grade water was also used

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as a negative control. The PCR reactions were performed using a thermocycler (Techne 3000G, BibbyScientific Ltd., UK) and using an initial denaturation at 95°C for 9 min followed by 40 cycles consisting of denaturation (95°C for 10 sec), annealing (47°C for 10 sec) and elongation (74°C for 10 sec) as their action conditions, and a final extension step at 78°C for 15 min. The PCR products were visualized using by a transilluminator (Kodak, Gel Logic 100, USA) after electrophoresis in 1,5 % agarose gels containing ethidium bromide (Figure 1).

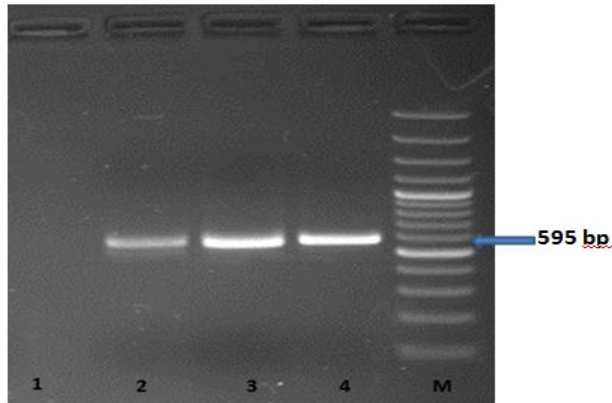


Fig 1: Ethidium bromide stained agarose gel electrophoresis of PCR products

[M: Marker (100 bp DNA ladder) 1: negative control (distilled water); 2,3,4: specific product for parapoxvirus].

DNA Sequencing :Parapoxvirus specific DNA in one of the samples was confirmed by sequence analysis (Beckman Coulter CEQ 8000, USA). Positive amplicon was sequenced commercially and the accession number of B2L gene sequence of strain in the GenBank was recorded as KX013765.

Biochemical study: Element analysis was continued with the plasma obtained from the complete blood samples of sheep with the diagnosis CE infection, and the control group.

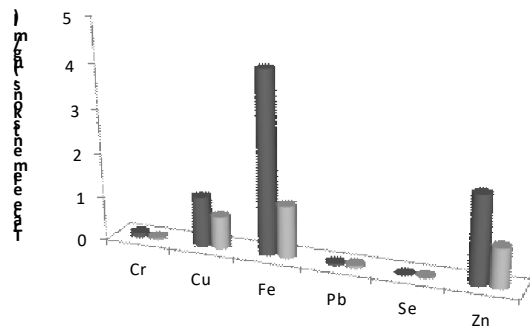


Fig 2: The change of the levels of trace elements (Cr, Cu, Fe, Se, Zn, Pb) in sheep with Contagious ecthyma and the control group (µg/mL). Black: Contagious ecthyma, Grey: Control group

The method of Papageorgiou *et al.* (2002) was used with some modifications. In the study, 1 mL of plasma was completed to 10 mL with 3% acid solution that was prepared from 65% HNO₃ (Merck, Germany) with bi-distilled water, and centrifuged at 3000xg for 15 minutes. The supernatant after the centrifugation was transferred into plastic tubes by adding 1 mL of 1% Triton-X, and analyzed by the inductively-coupled plasma-optic emission spectroscopy (ICP-OES Thermo scientific ICAP 6000 Series) (0.005ppm detectable limit) device. The levels of Be, Bi, Pb, Cd, Ca, Mg, Na, K, Fe, Cu, Zn, Se, Ni, Mn, Co, Cr, Li elements were investigated in the analysis.

Statistical analysis: Data were expressed as means±standard deviation (SD), and the differences between sample groups were evaluated using the Mann–Whitney U test. Significant differences between the two groups were considered when P was ≤0.05.

RESULTS AND DISCUSSION

The parapoxvirus infection in sheep, which were exemplified in the study, were identified by PCR. The elements Be, Bi, Pb, Cd, Na, K, Ca, Mg, Fe, Cu, Zn, Se, Ni, Mn, Co, Cr, Li were analyzed in the plasma of infected sheep with CE detected by PCR, and the control group. According to the results of the study, the elements Be, Bi and Cd, which are toxic, were not detected. In addition, the trace elements Ni, Mn, Co and Li could not be found in the existing sheep plasma.

In the study, the levels of all other trace elements Fe, Cu, Zn, Se, Cr, Pb except for Cr, were found to show

Table 1: The change of the levels of trace elements (Fe, Cu, Zn, Se, Cr, Pb) in sheep with Contagious ecthyma and the control group (µg/mL)

Elements	Contagious Ecthyma with sheep	Control group
Fe	1,190±0,244	4,107±1,843*
Cu	0,740±0,114	1,140±0,297*
Zn	0,899±0,347	1,981±0,766*
Se	0,013±0,001	0,028±0,021*
Cr	0,103±0,061	0,050±0,029
Pb	0,053±0,049	0,071±0,0274*

Mean±standard deviation. *P<0.05

Table 2: The change of the levels of trace elements (Na, K, Ca, Mg) in sheep with Contagious ecthyma and the control group (µg/mL)

Elements	Contagious Ecthyma with sheep	Control group
Na	2537,020±552,370	2910,925±384,206*
K	381,218±111,162	281,023±60,830
Ca	96,128±24,188	99,073±18,883
Mg	23,342±5,953	27,886±8,985

Mean±standard deviation. *P<0.05.

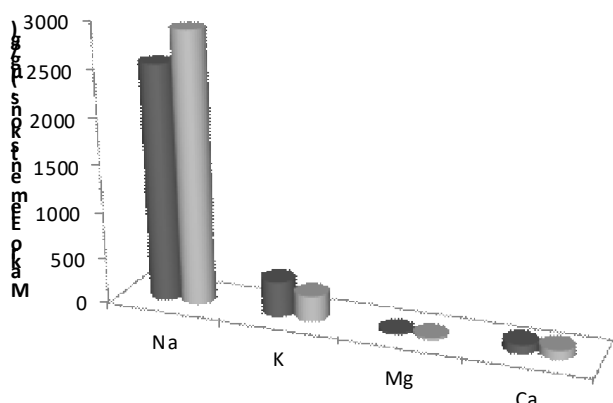


Fig 3: The change of the levels of trace elements (Na, K, Ca, Mg) in sheep with Contagious ecthyma and the control group ($\mu\text{g/mL}$). Black: Contagious ecthyma, Grey : Control group

statistically significant differences between the infected sheep with parapoxvirus and the control group (Table 1) (Fig.2) ($P < 0.05$).

The levels of elements Na, K, Ca, Mg of the macro elements were investigated. According to the results, a statistically significant difference was detected between the sheep with CE and the control group, with regard to the element Na (Table 2) (Fig.3) ($P < 0.05$).

Trace elements are very important elements that take part in many biochemical and metabolic reactions in the organism, and that are necessary for the immune system. The trace elements required by the animals show variations with age, the level of production, pregnancy and the lactation period. Macro and trace elements should be present in the organisms for normal growth and the continuity of life. The mineral levels in the metabolism are highly effective in the combat against bacterial, parasitic and viral infections (Wooldridge and Williams, 1993).

Contagious ecthyma, caused by parapoxvirus, constitutes one of the most common skin diseases in sheep. Ecthyma contagiosum infection, which is one of the major problems in terms of productivity in sheep breeding, is common in herds and may cause death, especially in lambs. In CE, the clinical findings help in the diagnosis of the disease; however, it may be confused with sheep pox virus, foot and mouth disease, bovine herpes virus type-2 and bluetongue virus infections (Inoshima *et al.*, 2002). Therefore, laboratory techniques are necessary for the accurate diagnosis. In the study, PCR was used for the diagnosis of parapoxvirus in sheep. PCR, which is used for the diagnosis of infectious diseases, was determined to be a rapid, sensitive and specific method in previous studies performed (Mazur *et al.*, 2000; Debnath *et al.*, 2016).

When the results of the sheep with CE and the control group were analyzed, the level of the trace elements was found to be lower in the infected sheep, and higher in the control group. In a research conducted in a mountainous region in China on healthy sheep serum, the levels of Cu, Mn, Fe, Zn elements were found as (0.2, 0.2, 7.0, 1.9 $\mu\text{g/mL}$), respectively (Wang, 2014). Furthermore, in studies in which the sheep samples were used by many researchers (Miedico *et al.*, 2016), the serum Cu levels were determined as 0.8 and 1.2 $\mu\text{g/mL}$, and the Zn levels as 6.9 and 14.8 $\mu\text{g/mL}$. The Cu levels found in this study were in line with these studies. When the Cu levels fall below levels of 0.5 $\mu\text{g/mL}$ in ruminants, the metabolism begins to be disordered (Zhou *et al.*, 2009). In this study, the Cu level results were determined to be higher than this rate. The level of Fe was seen to be much lower in the group infected with parapoxvirus, compared to the previous studies. Iron binding drugs were found to be highly effective in combating bacterial, parasitic and viral infections in the studies performed (Wooldridge and Williams, 1993).

In a study conducted by Naghadeh *et al.* (2015) in sheep, the plasma Se levels were found to vary between 0.01-0.29 ($\mu\text{g/mL}$). In a study conducted by Humann-Ziehank *et al.* (2008) in sheep, the serum Se and Zn levels were reported as (0.055 and 0.59 $\mu\text{g/L}$), respectively. In a study in which measurements of Se in sheep serum and cerebrospinal fluid (CSF) were carried out, the serum Se levels were found to be in the range of 1.24-21.6 (ng/mL) (Humann-Ziehank *et al.*, 2016). When the results of the Se levels of the presented study and other studies were compared, it was seen that in the CE group in particular, the Se levels were lower. The low results in the control group may give the idea that this condition could be associated with regional facts or due to the feed. In the absence of Se, which plays an important role in the antioxidant structure (such as GSH-Px), the immune system of the sheep may weaken. It may be considered that sheep with weakened immune system may become infected more easily with viral infections such as parapoxvirus infection. In a study ICP-MS with quadrupole in the sheep blood, the Cr levels were found to be between 0.3-0.05 ($\mu\text{g/mL}$). These values appear to be compatible with the Cr levels detected in this study (Batista *et al.*, 2009).

In a study conducted by Liu (2003), where the levels of metal in sheep and horses were compared, the blood levels of Pb, Zn, Cu were determined as 0.05, 10.6, 0.76 $\mu\text{g/L}$, respectively. Except for the Zn levels, all other values were seen to be consistent with the presented study results. In a study conducted by Smith *et al.* (2010), according to the seasons, the level of Pb in the sheep blood was observed to vary in the range of 3-275 ($\mu\text{g/mL}$).

The macro-mineral status measurements were carried out according to the seasonal changes in the sheep

blood in 4 different countries. In the study, the levels of Ca, K, Mg, Na elements were determined as 119-98, 246-175, 30-23, 3080-2840 µg/l, respectively (Xin *et al.*, 2011). These values seem to be consistent with the performed study. Of the macro elements, the levels of the element Na were seen to be low in sheep with CE and this difference appears to be statistically significant (Table 2). The low levels of the element Na, which is present in all body fluids, observed in sheep infected with parapoxvirus, may be explained with the loss of fluid, due to insufficient feeding. The results of Wu *et al.*'s (2013) study, in which they determined the nutrition of goats and the levels of macro elements, show parallelism with the levels of macro elements obtained in this study.

This study investigated the levels of metal concentrations in the control group and in sheep with CE.

Fe, Cu, Zn, Se, Cr and Pb the trace element in the sheep plasma were determined in the study. Of the macro elements, the concentration levels of Na, K, Ca and Mg elements were examined. Generally, the levels of metal concentrations in sheep infected with parapoxvirus were determined to be low. These results were generally consistent with the literature, or some were seen to be lower. This situation can be considered to be associated with regional facts or due to the feed. When there are low metal concentrations in sheep with CE, the immune system may be considered to be weakened. Further studies may be performed with regard to the increased risk of disease development as a result of the decreased levels of concentrations of Fe, Cu, Zn and Se in particular in sheep infected with the virus.

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