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Hematological and biochemical evaluation of goats naturally infected with contagious ecthyma

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ABSTRACT

Contagious ecthyma (CE) is a zoonotic skin disease of small ruminants, caused by an epitheliotropic parapoxvirus and has a worldwide distribution with significant economic importance. The objective of this study was to determine clinicopathlogic abnormalities in goats naturally infected with CE. Thirty two goats, 16 affected with CE and 16 normal healthy goats were used in this study. CE was confirmed by histopathology and PCR. Blood samples were collected from jugular veins for hematological and biochemical analysis. The PCV, WBC and neutrophil counts of CE affected goats were significantly higher than those in the unaffected goats ($p < 0.05$). Serum biochemical analysis revealed significantly higher levels of BUN, glucose, MDA and iron concentrations as well as CK, AST, GGT and catalase activities in CE affected goats than healthy animals ($p < 0.05$). The serum activity of catalase, SOD and GPx in goats with CE were significantly lower than those in normal goats. Creatinine concentration in serum of goats with CE was significantly lower than that in heathy ones ($p < 0.05$). There was no significant difference in serum total protein, albumin, total and direct bilirubin, and cholesterol concentrations between CE affected and healthy goats. The alterations observed in hematological and biochemical parameters of CE affected goats could be related to weight loss, subnutrition, oxidative stress and pathological changes including inflammation and secondary bacterial infection. These findings could be useful for the management of cases of sheep and goats with CE.

Keywords

Contagious ecthyma, Goats, Parapoxvirus, Orf, Clinical pathogy

Abbreviations

CE: contagious ecthyma
PCV: packed cell volume
WBC: white blood cells
EDTA: ethylenediaminetetraacetic
BUN: blood urea nitrogen
CK: creatine kinase
AST: aspartate amino transferase
HDL: high density lipoprotein
MDA: malondialdehyde
GPx: glutathione peroxidase
SOD: superoxide dismutase

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Introduction

Contagious ecthyma (CE) also known as contagious pustular dermatitis, Orf and ovine pustular dermatitis is a specific nonsystemic eruptive skin disease of domestic and wild small ruminants, mainly sheep and goats, which is caused by aepitheliotropic parapoxvirus or Orf virus, of the subfamily *Chordopoxvirinae*, family *Poxviridae*. Parapoxviruses include four species currently recognized by the International Committee on Taxonomy of Viruses: Orf virus, bovine popular stomatitis virus, Pseudocowpox virus and Parapoxvirus of red deer. CE disease has a worldwide distribution, prevalent in all countries where sheep and goats are raised and has a significant economical importance [1].The virus is highly resistant to desiccation in the environment, having been retained in dried crusts for long periods (up to 23 years) at cool temperature. In the laboratory, it is also resistant to glycerol and to ether, but very sensitive to treatment with chloroform [2].The disease also has a zoonotic potential as other parapoxviruses, although it is more of an occupational hazard to people working with animals, more often in farmers and veterinary staffs [3]. Humans acquire the infection from direct contact with infected, recently vaccinated animals and virus-contaminated fomites. The infection will stablish only at sites where skin is traumatized. Human CE occurs as one to a few self-limit lesions on the skin which can heal in 3-6 weeks [3, 4].

The lesions and clinical signs of CE in small ruminants are well described in the literature. In sheep and goats the disease is characterized by the development of scabby and proliferative lesions at the mucocutaneous junction of the lips and around erupting incisor teeth which may extend to the mucosa of the buccal cavity. Occasionally, lesions are found on the feet and around the coronet. In the flock where the disease occurs for the first time, morbidity rates can be up to 100% with a case-fatality rates of 5 to 15%. Mortality is common in young lambs and kids due to extension of lesions to the lower respiratory tract, starvation, secondary bacterial infection and cutaneous myiasis [1, 2].

Hematology and serum biochemical alteations have been demonstrated in a few numbers of goat diseases including mastitis [5], coccidiosis [6] and tick infestation [7].The propose of the study reported here was to determine the clinicopathologic abnormalities in goats affected with CE.

Results

The goats selected for sampling showed typical signs of CE including scab and crusty proliferation lesions on the lips (Figure 1). According to the history of the herd, the affected goats suffered from some restriction in grazing which had resulted to weight loss. Histopathology of the biopsy samples revealed a marked epidermal hyperplasia and hyperkeratosis. Keratinocytes of the stratum spinosum showed vacuolation and ballooning degeneration. Characteristic intracytoplasmic eosinophilic inclusion bodies were found in small numbers of keratinocytes of the lesions.

After PCR test for a scab material of CE lesion and sequencing of the PCR product, the blast of the read sequence revealed a maximum degree of sequence homology (100%) of the isolate with Orf Virus Strain OV-SA00. The result of agarose gel electrophoresis has been shown in Figure 2.

Hematological parameters and serum ferritin, transferrin and iron amounts obtained from goats naturally infected with CE and healthy goats are given in Table 1. PCV value, WBC, lymphocytes and neutrophils and serum iron concentrations of goats with CE were significantly higher than healthy goats ($p < 0.05$). There were no significant differences in serum ferritin and transferrin between goats with CE and heathy ones. Results of biochemical analyses of serum are shown in Table 2. Serum BUN, cholesterol, triglyceride, HDL, glucose and MDA concentrations as well as CK, AST, GGT and catalase activities were significantly higher in CE affected goats when compared with healthy goats ($p < 0.05$). Creatinine concentration in serum of affected goats with CE was significantly lower than those in healthy ones ($p < 0.05$). There was no

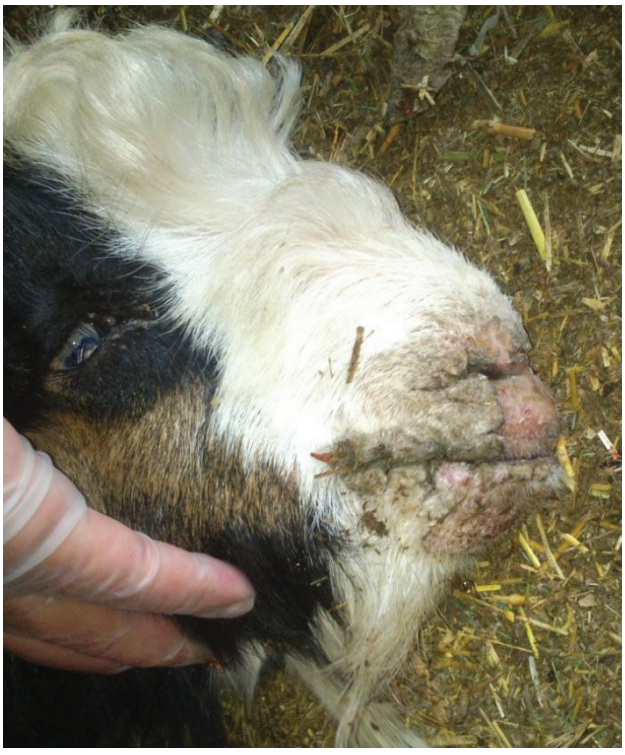

Figure 1
CE lesions on the oral commissure and muzzle of an affected goat.

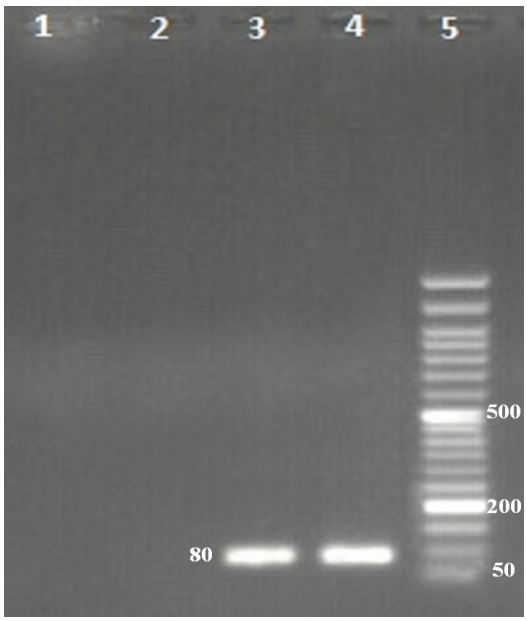

Figure 2
Detection of CE virus DNA using PCR test for scab materials of a CE infected goat.
Lane 1: negative control,
lane 3: positive control (80bp),
lane 4: positive sample (80bp)
lane 5: DNA size marker (50pb).

Table 1
Leukogram and serum Fe, ferritin and transferrin of goats naturally infected with CE.

	CE	Control
Parameters	**Mean ± SD**	**Mean ± SD**
Serum Iron (mg/dL)	79.077 ± 8.902	67.182 ± 11.557*
Ferritin (mg/dL)	3.000 ± 0.412	2.887 ± 0.203
Transferrin (mg/dL)	240.933 ± 25.769	236.000 ± 28.898
WBC count	7861.54 ± 2205.12	11444.44 ± 2092.62*
Lymphocytes	3013.67 ± 921.17	4150.00 ± 914.59*
Neutrophils	4564.00 ± 1181.46	6434.55 ± 1560.03*
Eosinophils	197.00 ± 41.23	365.00 ± 133.60
Monocytes	460.80 ± 104.82	370.00 ± 212.13
PCV (%)	26.929 ± 4.480	30.529 ± 3.676*

$p < 0.05$ from corresponding control

Table 2
Serum biochemical parameters of goats naturally infected with CE.

		Control
Parameters	**Mean±SD**	**Mean±SD**
Total protein (g/dL)	7.913 ± 0.354	7.707 ± 0.591
Albumin (g/dL)	3.731 ± 0.101	3.640 ± 0.241
Creatinine (mg/dL)	1.019 ± 0.142	0.700 ± 125*
Total bilirubin (mg/dL)	0.592 ± 0.0853	0.560 ± 0.0716
Direct bilirubin (mg/dL)	0.173 ± 0.0338	0.167 ± 0.0348
BUN (mg/dL)	37.500 ± 7.003	57.143 ± 9.984*
Cholesterol (mg/dL)	85.615 ± 8.752	77.091 ± 13.773
Triglyceride (mg/dL)	79.563 ± 5.366	88.545 ± 10.709
HDL (mg/dL)	20.200 ± 2.242	23.083 ± 3.343
CK (IU/L)	46.286 ± 17.670	91.000 ± 22.723*
AST(IU/L)	76.333 ± 6.985	102.125 ± 10.776*
GGT (IU/L)	49.923 ± 6.075	56.143 ± 7.284*
Catalase (U/mg protein)	43.586 ± 9.482	31.707 ± 8.532*
GPx	39.83 ± 8.625	30.31 ± 8.60*
SOD	33.58 ± 7.65	25.33 ± 8.69*
MDA (nmol/mg protein)	0.712 ± 0.167	1.981 ± 0.382*
Glucose	44.133 ± 5.383	56.231 ± 6.660*

*$p < 0.05$ from corresponding control

significant difference in serum total protein, albumin, total and direct bilirubin and cholesterol between CE affected and healthy goats.

Discussion

Although CE as a viral disease affecting the skin primarily around the mouth, udder and coronet, it causes considerable devastating economic losses mainly due to the long lasting nature of the outbreaks, mortality in neonates and reduction of performance in affected animals [2]. To the authors' knowledge serum biochemical and hematological references of goats naturally infected with CE has not been previously reported, and the reported clinical pathology of the disease was almost focused on the diagnostic procedures including PCR methods [1, 2].The measurement and evaluation of the biochemical profile may be helpful in elucidating the pathogenesis and control of the disease.

Although CE is generally considered a non-systemic disease in small ruminants [1], results of the present study revealed noticeable hematological and serum biochemical alterations in affected goats.

According to the comparative hematological analysis, based on PCV, WBC and cell differentiation, the CE affected goats presented higher levels of those parameters than those in animals without CE.These hematological changes might

be related to animal response to CE inflammatory lesions particularly to secondary bacterial infection. Also stress condition caused by CE could lead to such alterations. Higher levels of serum glucose may also confirm the effect of stress in goats with CE. CE affected goats in this study had significantly higher serum CK and AST activities. This finding is in agreement with those reported in cattle infected with lumpy skin disease, a viral disease with skin lesions caused by a parapoxvirus [8, 9]. The significant increase of CK and AST in CE infected goats could be due to muscle damage involvement. Serum elevation of AST may also be associated to liver damage and its function disturbances, however other biochemical parameters including bilirubin did not indicate any liver dysfunction in goats with CE in the present report. In domestic animals serum GGT activity is derived solely from the liver and its elevation is associated with the damage of the biliary epithelial cells [10]. Because of no indication of liver damage in CE affected goats in the present report, higher levels of GGT activity in these animals in unknown and can be considered as an unexpected finding. It has also been suggested that GGT could be a consistent cellular-biochemical marker of stress responsiveness due to its activity in different lymphoid tissues of rats [11].

Significantly lower levels of serum creatinine in CE affected goats may be related to the body weight loss because of undernutrition status during the course of the disease. Varying degrees of pains of oro-labial lesions in cases of CE which interferes with animal grazing leads to unthriftiness and economic losses. Changes in lipid profile may also be associated to that nutritional status[1].

In the present report, it is demonstrated that CE infection increases lipid peroxidation end products which was indicated by the elevation of the MDA concentration in the serum of affected animals. Also, this was associated with reduction of antioxidant enzymes activity, catalase, SOD and GPx in the serum. These evidence suggest the oxidative stress occurrence during the CE infection in goats. Polyunsaturated lipids are susceptible substrates to free radical oxidative damage and biomarkers of lipid peroxidation including MDA, are considered the best marker of oxidative stress [12]. Oxidation and the production of free radicals are an integral part of aerobic metabolism. A variety of reactive oxygen species (ROS) are produced during normal metabolic processes in all living organisms including certain leukocyte populations when defense against disease. High levels of ROS is harmful for the cells and tissues. If these compounds are not removed by endogenous antioxidants, the rate of oxidation will exceed the rate of anti-oxidation and leads to oxidative stress and eventual damages of macromolecules [13, 14]. Signs of this event observed in the present study in serum of goats affected with CE were significantly higher concentration of MDA and lower catalase activity. Development of oxidative stress has been reported in sheep naturally infected with pox virus [15], and in goats infected with mycoplasma agalactiae [16]. Both acute and latent herpes virus-1 infections in the murine nervous system are associated with oxidative damage [17, 18]. It is suggested that infectious diseases are associated with oxidative stress in a number of ways including inflammation, organ damage combined with altered metabolism and overloading of iron [19, 20]. The alteration observed in hematological and biochemical parameters of CE affected goats could be related to weight loss, subnutrition, oxidative stress and pathological changes including inflammation and secondary bacterial infection. These findings could be useful for the management of cases of sheep and goats with CE.

Materials and methods

During an outbreak of CE in Shahrekord district in Iran, 16 native goats aging 1-3 years were examined clinically in a herd for the presence of CE lesions in July 2016. Blood was collected by puncture of the jugular vein from goats into 2 samples. The first blood sample was taken into ethylenediaminetetraacetic (EDTA) tubes for hematological analysis. The second blood sample was taken in a sterile test tube for separation of serum that was used for biochemical measurements. A biopsy sample and scab materials of CE lesion were also taken for confirmation of the disease by PCR test and histopathology. The biopsy sample was fixed in buffered formalin and processed for histopathology. The scab material was processed for Orf virus DNA extraction and PCR. Electrophoresis of the PCR product on the agarose gel (1.8%) and subsequent sequencing of the positive PCR product performed to confirm the presence of OrfV genome in the lesion.

The presence of OrfV was detected using the primers designed by Beacon designer software. The sequences of the Forward and reverse primers were 5'- TTCAACGGCCACAACTTCG -3' and 5'- GCCGAAAGCGGATGTGCTC -3', respectively. Each PCR reaction was performed in a final volume of 25 µl containing 11 µl of deionized sterile water, 10 µl of Taq DNA Polymerase 2x Mix Red-Mgcl$_2$ 2 mM (GeneAll, Cat. no. A180301), 1 pmol of each primer and 2 µl of DNA template.

The thermal cycling conditions for the amplification were 1 cycle for 3 min at 94 ˚C, 30 cycles of 30 s at 94 ˚C, 30 s at 57 ˚C and 30 s at 72 ˚C, with a final extension step of 10 min at 72 ˚C. Positive and negative controls (a previously sequenced positive sample of OrfV and water, respectively) were included in each analysis. 6 µl of the amplified products were loaded on a 1.8% agarose gel, and visualized by staining with DNA Staining Dye (Green Viewer™, Cat No: B111151) and compared to DNA markers (50 base pair ladder, Fermentas). Then, the PCR positive sample in a volume of 50 µl were sent to Bioneer Company (South Korea) for sequencing.

Sixteen clinically healthy goats from another herd of the same district were sampled and used as the control group. Blood samples without anticoagulant were centrifugated at 3000 rpm for 10 min for serum separation and stored at –70°C until testing.

Hematological parameters including packed cell volume (PCV) value, white blood cells (WBC) and differential leukocyte counts were measured by routine procedures. Biochemical anal-

yses of serum were performed using an autoanalyzer (I.S.E., Italy) and commercial kits (Pars Azmoon, Tehran, Iran). Creatine kinase (CK), aspartate aminotransferase (AST) activity, blood urea nitrogen (BUN), gamma glutamyltransferase (GGT), total protein, albumin, creatinine, glucose, triglyceride, high density lipoprotein (HDL), cholesterol, total bilirubin, direct bilirubin, iron, ferritin and transferrin levels were analyzed. The intra and inter-assay coefficients of variations for CK were 0.70% and 1.00%, for AST 3.06% and 1.38%, for GGT 1.16% and 0.97%, for total protein 0.90% and 1.06%, for albumin 1.12% and 1.44%, for creatinine 3.38% and 0.87%, for glucose 1.74% and 1.19%, for triglyceride 1.82% and 1.60%, for HDL 0.73% and 1.80%, for cholesterol 0.61% and 1.22%, for total bilirubin 2.32% and 3.49%, for direct bilirubin 1.46% and 1.00% and for Iron 1.22% and 2.19%, respectively.

The serum catalase activity was assayed according to the method of Goth [21]. (1991). The activity of superoxide dismutase (SOD) was measured by nitrobluetetrazolium (NBT). In this method the superoxide radicals onvert NBT to a blue-colored $NBTH_2$ [22]. Glutathion peroxidase (GPx) was determined using the method described by Paglia and Valentine [23]. Serum malondialdehyde (MD) concentrations, also known as thiobarbituric acid reactive substances (TBARS), were determined colorimetrically using the method of Buege and Aust [24]. (1978).

Statistical Analysis. The results were expressed as mean ± standard deviations. Differences between groups were determined using *t*-test, and $p < 0.05$ was considered to be statistically significant. Shapiro-Wilks Normality Test was used to detect normal distribution of data. All statistical analysis were performed by Sigma-plot 13.

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Author Contributions

Conceived and designed the experiments: MR. A., Performed the experiments: SA.KA., Analyzed the data: A.M., A.M. Wrote the paper: MR.A., A.M.

Conflict of Interest

None.

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