



## Research Article

# Investigation of Matrix Metalloproteinase-2 and Matrix Metalloproteinase-9 Activity in Some Inflammatory Conditions in Barki Sheep

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**Abstract** | Inflammation is a double-edged weapon. Although it starts as a protective mechanism, it ends with serious clinicopathological alterations, which disappoint our therapeutic programs and frustrate our expectation for curing. This work aimed to assess the most important clinicopathological alterations related to sheep pneumonia, arthritis and enteritis and spot the light on MMP-2 and MMP-9 diagnostic and prognostic accuracy in these conditions. 80 barki lambs were divided into four groups control group (CG), pneumonic group (PG), arthritic group (AG) and enteric group (EG). Blood samples were collected from the four groups then haematologically, biochemically and statistically analyzed. A significant ( $P < 0.05$ ) microcytic hypochromic anemia was observed in the PG and AG while, EG suffered from a macrocytic hyperchromic anemia. On contrast, a neutrophilic leukocytosis and lymphocytopenia was described in the three diseased groups. Biochemically, a significant ( $P < 0.05$ ) increase in total protein, globulin, liver enzymatic activities, kidney function tests, total lipids, triglycerides, MMP-2 and MMP-9 concentrations was depicted in the three diseased groups. On the contrary, the total cholesterol, HDL-cholesterol, LDL-cholesterol, minerals, electrolytes, trace elements and total antioxidants capacity concentrations significantly ( $P < 0.05$ ) decreased in the three diseased groups. Both of MMP-2 and MMP-9 yielded a sensitivity and NPV as 100% in the sheep pneumonia, arthritis and enteritis and correlated with some parameters in PG and EG. Ovine inflammatory conditions usually result in different clinicopathological changes. Monitoring of them may be useful in improving our therapeutic intervention and enhancing the animal response to treatment. Both of MMP-2 and MMP-9 is a good marker for sheep inflammatory conditions.

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**Key words** | Sheep, Pneumonia, Arthritis, Enteritis, Matrix metalloproteinases (2-9), clinicopathological changes.

## Introduction

Barki sheep breeding is an integral part of the Bedouin life in the Egyptian North West coast. Beside its economic importance as a source of meat and wool, it has a socio-religious value (Othmana et

al., 2016; Donia et al., 2018). Unfortunately, different inflammatory conditions attack sheep flocks in large scales leading to high mortalities, poor growth rates, low carcass yield and high condemned organs percentage. The high cost of prophylactic and therapeutic programs are another serious economic

losses added to the previous losses (Zein-Eldin et al., 2013; Jesse et al., 2017; Donia et al., 2018). Surely, absence of good knowledge about the hematological and biochemical changes associated with different ovine inflammatory conditions as well as late detection of them partially participate in augmentation of the prior economic losses.

MMP-2 and MMP-9 are two proteolytic enzymes, classified under a super family of Zn containing endopeptidases, called matrix metalloproteinases (MMPs). Generally, MMPs are responsible for extracellular matrix turnover and cleavage while, MMP-2 and MMP-9 are mainly specialized in gelatin degradation so called gelatinases. In addition to gelatin, they are capable of collagens, elastin and vitronectin degeneration (Garg et al., 2006; Meszaros and Malemud, 2012; Li et al., 2016). Physiologically, they are embedded in various processes such as angiogenesis, endothelial cells apoptosis, vascular basement membrane degradation thus facilitating cellular infiltration and pro-inflammatory mediators stimulation and suppression. They are strictly controlled by tissue inhibitor of metalloproteinases (TIMPs). The imbalance between MMPs and TIMPs is tightly connected to a large section of diseases pathogenesis particularly malignancy and inflammation (Strup-Perrot et al., 2004; Gao et al., 2005). In spite of their curial importance in human medicine either as sensitive markers or therapeutic targets for different diseases, they are still little unknown in veterinary medicine (Meszaros and Malemud, 2012; Li et al., 2016).

Hence, this research aimed to monitor the most important clinicopathological changes associated with three major inflammatory conditions (pneumonia, arthritis, enteritis) in barking sheep and focus on MMP-2 and MMP-9 diagnostic and prognostic accuracy and their relation with other clinicopathological changes in these conditions.

## Material and Methods

According to the ethical guidelines of animal treatment and after the owners' agreements, blood samples were collected sporadically from 80 female barking lambs (6 months age) from Matrouh governorate different cities.

They were divided into 4 groups:

**Control group (CG):** 20 apparently healthy lambs (normal appetite, posture, body weight, plus (80-100

beat/min), temp (39-40°C) and respiration (36-40 breath/min), no nasal or ocular discharge (The Merck Veterinary Manual, 2006).

**Pneumonic group (PG):** 20 lambs suffered from respiratory manifestation (emaciation, loss of appetite, dyspnea, stretched head and neck, hyperthermia (41-42°C), nasal discharge, wheezes and crackles on auscultation, rapid abdominal respiration (41-46 breath/min)).

**Arthritic group (AG):** 20 lambs suffered from lameness (in most cases the animal carried the affected leg), swollen knee some times, hyperthermia (41-42°C), pain and anorexia. Enteric group (EG): 20 lambs suffered from diarrhea, pain, off food, low weight, dullness, depression and fever (41-42°C).

**Blood samples were collected from all lambs by jugular vein puncture, and then divided in two parts:** Potassium EDTA salt solution was added to the first part to stop the coagulation cascade and this part was used immediately for hematological parameters assessment according to Feldman et al. (2000) method. While, the second part was let to coagulate and was centrifugated at 3000 RPM. for 20 minutes at 37 °C, to get serum which used for estimation of different biochemical parameters spectrophotometrically by using Biodiagnostic company® commercial kits, Cairo, Egypt and serum MMPs concentrations by using Cloud- Clone Corp company® ELISA kits, Huston, USA (all manual instructions were taken in consideration).

## Statistical analysis

SPSS® program version 23 was used to evaluate the differences between the estimated parameters means (one-way ANOVA test), the post hoc differences between means (a multiple comparison Tukey's HSD test) and the correlations between the selected parameters (Pearson's simple correlation method). A difference was considerable significant at  $P < 0.05$ .

Graphed prism version 5 program was used to evaluate the cut off points, sensitivity, specificity and likelihood ratio (LR) for the measured MMPs between PG, AG, EG and CG.

The positive predictive value (PPV), negative predictive value (NPV) and accuracy rate for them were calculated according the next equations:

$$PPV = \text{True positive} \div \text{Total positive} \times 100$$

$$NPV = \text{True negative} \div \text{Total negative} \times 100$$

$$\text{Accuracy rate} = (\text{True positive} + \text{True negative}) \div \text{Total population} \times 100$$

$$\text{Percentage of increase} =$$

$$(\text{The mean value of the marker concentration in PG or AG or EG} -$$

$$\text{The mean value of its concentration in CG}) \div$$

$$\text{The mean value of its concentration in CG} \times 100$$

## Results and Discussion

**Table 1** revealed that a significant ( $P < 0.05$ ) decrease in the red cell parameters (RBCs, Hb, PCV, MCV, MCH, MCHC) between PG, AG and CG, but these parameters significantly ( $P < 0.05$ ) increased in EG in relation to CG, PG and AG. On contrast, a significant ( $P < 0.05$ ) neutrophilic leukocytosis and lymphocytopenia were detected between PG, AG, EG and CG.

**Table 2** showed a significant ( $P < 0.05$ ) hyperproteinemia, hyperglobulinemia, decreased A/G in PG, AG, EG when compared to CG. While, the albumin and glucose concentrations significantly ( $P < 0.05$ ) decreased in PG, AG in relation to CG and significantly ( $P < 0.05$ ) increased in EG in relation to CG.

A significant ( $P < 0.05$ ) decrease was observed between PG, AG, EG and CG in the total cholesterol, HDL-cholesterol, LDL-cholesterol, minerals (Ca, Mg, P), electrolytes (Cl, Na), trace elements (Cu, Zn) and total antioxidants capacity (TAC) concentrations. While K significantly ( $P < 0.05$ ) increased in PG in relation to CG, AG, EG and significantly ( $P < 0.05$ ) decreased in AG, EG in relation to CG. On the other hands, total lipids, triglycerides, the liver enzymatic activities (AST, ALT, ALP), kidney function tests (blood urea, Creatinine (Cr)), MMP-2 and MMP-9 significantly ( $P < 0.05$ ) increased between PG, AG, EG and CG.

The comparison between the three diseased groups exhibited a significant ( $P < 0.05$ ) raise in TLC, lymphocytes, total protein, albumin, glucose, total lipids, triglycerides, total cholesterol, HDL-cholesterol, minerals (Mg, P), Na, trace elements (Cu, Zn), total antioxidants capacity (TAC), liver enzymatic activities, kidney function tests, MMP-2 and MMP-9 in EG when compared to PG and AG. While, a significant ( $P < 0.05$ ) hyperglobulinemia was detected between EG and AG only.

In connection with the assessment of MMP-2 and MMP-9 importance as markers for the ovine

pneumonia, arthritis and enteritis, **Table 3** cleared that both of them yielded a sensitivity and NPV as 100% in sheep pneumonia, arthritis and enteritis. While, MMP-9 had higher values of specificity, LR, PPV and accuracy rate than MMP-2 but the percentage of increase was in the sake of MMP-2. The difference between the three groups showed that the PPV and accuracy rate for MMP-2 and MMP-9 were higher in PG than the other two groups. On the other hand, the highest percentage of increase was reported in EG followed by AG then PG. Meanwhile, **Table 4** illustrated a significant ( $P < 0.05$ ) positive correlation between MMP-2 and TP in PG. Meanwhile, there was a significant ( $P < 0.05$ ) positive correlation between MMP-9 and TLC, glucose, AST as well as a significant ( $P < 0.05$ ) negative correlation between MMP-9 and Zn, Mg, Na and Cu, but MMP-2 significantly ( $P < 0.05$ ) positively correlated with neutrophils and glucose and significantly ( $P < 0.05$ ) negatively correlated with Na levels in the EG. There was no significant correlations between MMP-2 and MMP-9 and other clinicopathological alterations in AG.

Inflammation is a double-faced process, arises primarily during different diseases courses as a defensive mechanism ([Nahed et al., 2016](#); [Jesse et al., 2017](#)). Regrettably, it usually leaves behind a mess of serious hematological as well as biochemical alterations. Concerning the hematological changes, the present study revealed that both of ovine pneumonia and arthritis accompanied with a typical microcytic hypochromic anemia. Basically, this anemia originated as a part of the protective inflammatory process. As the invigorated pro-inflammatory cytokines stimulate the hepcidin secretion which minimizes the dietary iron absorption from intestine and maximizes iron storage as ferritin and hemosiderin. Thus, prevent the invading microorganisms from getting the iron necessary for their growth and multiplication. By the time, this mechanism results in the outstanding anemia ([Nahed et al., 2016](#); [Jesse et al., 2017](#); [Donia et al., 2018](#)). In addition, these pro-inflammatory cytokines hinder the erythropoiesis through inhibition of erythropoietin and stem cell factor expression and decrease erythrocytes age ([Nahed et al., 2016](#); [Jesse et al., 2017](#)). Furthermore, they stimulate the massive production of different free radicals causing the oxidative stress increment and the subsequent red blood cell destruction and dysfunction ([Garcia-Gonzalez et al., 2015](#); [Allam et al., 2017](#)). On contrast, the detected increase in the red blood cell parameters in

**Table 1:** Comparison between the hematological parameters of the studied groups. Values are means  $\pm$  SD.

Parameter	CG	PG	AG	EG
RBCs ( $\times 10^6/\mu\text{l}$ )	12.59 $\pm$ 0.11 <sup>d</sup>	8.59 $\pm$ 0.29 <sup>a</sup>	8.64 $\pm$ 0.29 <sup>a</sup>	14.11 $\pm$ 0.07 <sup>a,b,c</sup>
Hb (g/dl)	14.18 $\pm$ 0.23 <sup>d</sup>	8.03 $\pm$ 0.06 <sup>a</sup>	8.03 $\pm$ 0.07 <sup>a</sup>	16.94 $\pm$ 0.03 <sup>a,b,c</sup>
PCV (%)	33.15 $\pm$ 0.81 <sup>d</sup>	20.17 $\pm$ 0.11 <sup>a</sup>	20.15 $\pm$ 0.11 <sup>a</sup>	38.27 $\pm$ 0.24 <sup>a,b,c</sup>
MCV (fl)	26.32 $\pm$ 0.62 <sup>d</sup>	23.49 $\pm$ 0.80 <sup>a</sup>	23.35 $\pm$ 0.81 <sup>a</sup>	27.12 $\pm$ 0.22 <sup>a,b,c</sup>
MCH (pg)	11.25 $\pm$ 0.23 <sup>d</sup>	9.36 $\pm$ 0.34 <sup>a</sup>	9.30 $\pm$ 0.29 <sup>a</sup>	12.01 $\pm$ 0.07 <sup>a,b,c</sup>
MCHC (%)	42.78 $\pm$ 1.07 <sup>d</sup>	39.84 $\pm$ 0.38 <sup>a</sup>	39.85 $\pm$ 0.41 <sup>a</sup>	44.27 $\pm$ 0.26 <sup>a,b,c</sup>
TLC ( $\times 10^3/\mu\text{l}$ )	7.21 $\pm$ 0.13 <sup>d</sup>	10.73 $\pm$ 0.18 <sup>a</sup>	10.75 $\pm$ 0.35 <sup>a</sup>	11.18 $\pm$ 0.20 <sup>a,b,c</sup>
Neutrophils ( $\times 10^3/\mu\text{l}$ )	2.15 $\pm$ 0.02 <sup>d</sup>	7.12 $\pm$ 0.17 <sup>a</sup>	7.07 $\pm$ 0.30 <sup>a</sup>	7.14 $\pm$ 0.03 <sup>a</sup>
Lymphocytes ( $\times 10^3/\mu\text{l}$ )	4.06 $\pm$ 0.07 <sup>d</sup>	2.64 $\pm$ 0.22 <sup>a</sup>	2.71 $\pm$ 0.28 <sup>a</sup>	3.09 $\pm$ 0.06 <sup>a,b,c</sup>
Monocytes ( $\times 10^3/\mu\text{l}$ )	0.44 $\pm$ 0.07	0.46 $\pm$ 0.05	0.46 $\pm$ 0.05	0.42 $\pm$ 0.07
Eosinophils ( $\times 10^3/\mu\text{l}$ )	0.53 $\pm$ 0.07	0.46 $\pm$ 0.05	0.46 $\pm$ 0.05	0.50 $\pm$ 0.09
Basophils ( $\times 10^3/\mu\text{l}$ )	0.04 $\pm$ 0.05	0.05 $\pm$ 0.05	0.05 $\pm$ 0.05	0.03 $\pm$ 0.04

<sup>a</sup>(significant with CG); <sup>b</sup>(significant with PG ); <sup>c</sup>(significant with AG ); <sup>d</sup>(significant between the four groups); considered statistically significant at  $P < 0.05$

**Table 2:** Comparison between the biochemical parameters of the studied groups. Values are means  $\pm$  SD.

Parameter	CG	PG	AG	EG
Total protein (g/dl)	6.52 $\pm$ 0.24 <sup>d</sup>	7.42 $\pm$ 0.22 <sup>a</sup>	7.39 $\pm$ 0.24 <sup>a</sup>	8.89 $\pm$ 0.06 <sup>a,b,c</sup>
Albumin (g/dl)	3.99 $\pm$ 0.20 <sup>d</sup>	3.13 $\pm$ 0.58 <sup>a</sup>	3.40 $\pm$ 0.51 <sup>a</sup>	4.29 $\pm$ 0.51 <sup>a,b,c</sup>
Globulin (g/dl)	2.53 $\pm$ 0.30 <sup>d</sup>	4.29 $\pm$ 0.59 <sup>a</sup>	3.99 $\pm$ 0.49 <sup>a</sup>	4.60 $\pm$ 0.09 <sup>a,c</sup>
A/G	1.61 $\pm$ 0.27 <sup>d</sup>	0.76 $\pm$ 0.24 <sup>a</sup>	0.87 $\pm$ 0.21 <sup>a</sup>	0.93 $\pm$ 0.05 <sup>a</sup>
Glucose (mg/dl)	92.90 $\pm$ 2.07 <sup>d</sup>	75.23 $\pm$ 2.16 <sup>a</sup>	74.85 $\pm$ 1.98 <sup>a</sup>	122.30 $\pm$ 1.78 <sup>a,b,c</sup>
Total lipids (mg/dl)	355.40 $\pm$ 9.53 <sup>d</sup>	356.40 $\pm$ 10.72	359.47 $\pm$ 13.85	373.28 $\pm$ 5.38 <sup>a,b,c</sup>
Triglycerides (mg/dl)	73.17 $\pm$ 2.12 <sup>d</sup>	126.50 $\pm$ 2.56 <sup>a</sup>	127.06 $\pm$ 2.95 <sup>a</sup>	135.82 $\pm$ 3.77 <sup>a,b,c</sup>
Phospholipids (mg/dl)	161.04 $\pm$ 8.88	159.47 $\pm$ 9.42	161.79 $\pm$ 10.66	162.88 $\pm$ 2.02
Cholesterol (mg/dl)	121.19 $\pm$ 1.98 <sup>d</sup>	70.43 $\pm$ 3.34 <sup>a</sup>	70.62 $\pm$ 3.49 <sup>a</sup>	74.58 $\pm$ 3.63 <sup>a,b,c</sup>
HDL-cholesterol(mg/dl)	86.90 $\pm$ 1.39 <sup>d</sup>	46.21 $\pm$ 1.72 <sup>a</sup>	46.31 $\pm$ 1.74 <sup>a</sup>	49.50 $\pm$ 2.25 <sup>a,b,c</sup>
LDL-cholesterol (mg/dl)	34.29 $\pm$ 1.40 <sup>d</sup>	24.21 $\pm$ 1.72 <sup>a</sup>	24.31 $\pm$ 1.74 <sup>a</sup>	25.08 $\pm$ 2.70 <sup>a</sup>
Ca (mg/dl)	11.01 $\pm$ 0.27 <sup>d</sup>	8.76 $\pm$ 0.28 <sup>a</sup>	8.77 $\pm$ 0.31 <sup>a</sup>	8.94 $\pm$ 0.05 <sup>a</sup>
P (mg/dl)	6.35 $\pm$ 0.27 <sup>d</sup>	3.77 $\pm$ 0.53 <sup>a</sup>	3.79 $\pm$ 0.51 <sup>a</sup>	4.87 $\pm$ 0.05 <sup>a,b,c</sup>
Cl (mmol/L)	105.23 $\pm$ 2.64 <sup>d</sup>	89.79 $\pm$ 4.31 <sup>a</sup>	90.06 $\pm$ 4.91 <sup>a</sup>	92.94 $\pm$ 1.71 <sup>a,b</sup>
Na (mmol/L)	142.40 $\pm$ 2.80 <sup>d</sup>	108.93 $\pm$ 0.66 <sup>a</sup>	108.97 $\pm$ 0.70 <sup>a</sup>	126.61 $\pm$ 3.56 <sup>a,b,c</sup>
K (mmol/L)	3.47 $\pm$ 0.18 <sup>d</sup>	6.05 $\pm$ 0.13 <sup>a</sup>	2.82 $\pm$ 0.39 <sup>a,b</sup>	2.90 $\pm$ 0.04 <sup>a,b</sup>
Mg (mg/dl)	3.71 $\pm$ 0.50 <sup>d</sup>	2.66 $\pm$ 0.04 <sup>a</sup>	2.66 $\pm$ 0.04 <sup>a</sup>	2.97 $\pm$ 0.04 <sup>a,b,c</sup>
Cu ( $\mu\text{mol/L}$ )	23.55 $\pm$ 1.31 <sup>d</sup>	16.72 $\pm$ 0.26 <sup>a</sup>	16.72 $\pm$ 0.28 <sup>a</sup>	20.59 $\pm$ 0.72 <sup>a,b,c</sup>
Zn ( $\mu\text{g/dl}$ )	155.72 $\pm$ 7.65 <sup>d</sup>	127.82 $\pm$ 3.34 <sup>a</sup>	127.57 $\pm$ 3.51 <sup>a</sup>	133.93 $\pm$ 6.73 <sup>a,b,c</sup>
TAC (Mm/L)	1.23 $\pm$ 0.11 <sup>d</sup>	0.89 $\pm$ 0.05 <sup>a</sup>	0.90 $\pm$ 0.05 <sup>a</sup>	0.70 $\pm$ 0.04 <sup>a,b,c</sup>
AST (U/L)	26.74 $\pm$ 1.61 <sup>d</sup>	33.41 $\pm$ 1.41 <sup>a</sup>	33.13 $\pm$ 1.32 <sup>a</sup>	37.01 $\pm$ 1.38 <sup>a,b,c</sup>
ALT (U/L)	36.74 $\pm$ 1.61 <sup>d</sup>	43.42 $\pm$ 1.72 <sup>a</sup>	43.36 $\pm$ 1.68 <sup>a</sup>	46.86 $\pm$ 1.40 <sup>a,b,c</sup>
ALP (U/L)	28.54 $\pm$ 0.30 <sup>d</sup>	40.19 $\pm$ 1.57 <sup>a</sup>	40.33 $\pm$ 1.52 <sup>a</sup>	43.54 $\pm$ 1.44 <sup>a,b,c</sup>
Blood urea (mg/dl)	24.74 $\pm$ 0.73 <sup>d</sup>	38.14 $\pm$ 4.94 <sup>a</sup>	38.27 $\pm$ 5.38 <sup>a</sup>	44.68 $\pm$ 0.76 <sup>a,b,c</sup>
Cr (mg/dl)	0.75 $\pm$ 0.11 <sup>d</sup>	1.20 $\pm$ 0.21 <sup>a</sup>	1.19 $\pm$ 0.18 <sup>a</sup>	1.75 $\pm$ 0.11 <sup>a,b,c</sup>
MMP-2 (ng/ml)	15.39 $\pm$ 0.75 <sup>d</sup>	61.32 $\pm$ 1.14 <sup>a</sup>	61.18 $\pm$ 1.11 <sup>a</sup>	65.46 $\pm$ 0.89 <sup>a,b,c</sup>
MMP-9 (ng/ml)	22.75 $\pm$ 1.07 <sup>d</sup>	66.69 $\pm$ 2.96 <sup>a</sup>	67.63 $\pm$ 3.17 <sup>a</sup>	71.84 $\pm$ 3.18 <sup>a,b,c</sup>

<sup>a</sup>(significant with CG); <sup>b</sup>(significant with PG ); <sup>c</sup>(significant with AG ); <sup>d</sup>(significant between the four groups); considered statistically significant at  $P < 0.05$ .

**Table 3:** *Cut off points (ng/ml), sensitivity, specificity, LR, PPV, NPV, accuracy rate and percentage of increase for MMP-2 and MMP-9 in PG, AG, EG compared to CG.*

Statistical parameter	PG		AG		EG	
	MMP-2	MMP-9	MMP-2	MMP-9	MMP-2	MMP-9
Cut off	15.9	23.5	15.9	23.5	15.9	23.5
Sensitivity	100%	100%	100%	100%	100%	100%
Specificity	70%	75%	70%	75%	70%	75%
LR	3.33	4	3.33	4	3.33	4
PPV	83.33%	85.71%	76.92%	80%	76.72%	80%
NPV	100%	100%	100%	100%	100%	100%
Accuracy rate	88%	90%	85%	87.5%	85%	87.5%
% of increase	298.44%	193.14%	297.53%	197.27%	325.34%	215.78%

EG is fundamentally attributed to the excess fluid loss and related haemocncentration (Zein-Eldin et al., 2013). While, the remarkable neutrophilic leukocytosis noticed in the three diseased groups, is another outcome for the pro-inflammatory cytokines activation and dependant stimulation of the bone marrow production of the leukocytes especially neutrophils which are the first line of defense against pathogenic microorganisms (Zein-Eldin et al., 2013; Nahed et al., 2016; Jesse et al., 2017). On the opposite side, the lymphocytopenia registered in PG, AG, EG in the present work may be assigned to the lymphocytes migration to the infected organs (lung, joint, intestine) in order to prevent the infection expansion until the developing of specific immunity (Kumar et al., 2015; Nahed et al., 2016; Hassanein et al., 2017).

Regarding the biochemical changes, the protein profile of the pneumonic, arthritic, enteric lambs here presented a hyperglobulinemia followed by a hyperproteinemia and decreased A/G ratio. This hyperglobulinemia referred to immunoglobulins formation (humeral immunity) as well as release of innate immunity components (acute phase proteins, cytokines, MMPs) to limit the infective microorganisms propagation and spread as well as destroy them (Zein-Eldin et al., 2013; Nahed et al., 2016; Jesse et al., 2017). On the other hand, the recorded hypoalbuminemia in PG and AG was because of the acute phase response and the oxidative stress related to the inflammatory process. Whereas, albumin is a potent negative acute phase reactant and antioxidant in the same time (Zein-Eldin et al., 2013; Nahed et al., 2016; Jesse et al., 2017). Rationally, the hyperthermia and dependent anorexia (which are basic parts from the inflammatory

disease pathogenesis) are extremely involved in the outstanding hypoglycemia and hypoalbuminemia in PG and AG as well as hypocholesterlema, HDL-hypocholestelema, LDL-hypocholesterlema, hypocalcemia, hypophosphatemia, hypomagenesemia, hyponateremia, hypocholemedemia, hypozincemia and hypocupremia in PG, AG and EG and hypokalemia in both of AG and EG (Zein-Eldin et al., 2013; Joshi et al., 2015; Nahed et al., 2016; Bozukluhan et al., 2017; Jesse et al., 2017; Rodríguez-Carrio et al., 2017; Donia et al., 2018). Unlike AG and EG, PG showed a marked hyperkalemia whereas the blood buffering system responds to the respiratory acidosis (mostly accompanied the pneumonia cases) via pumping the excess H<sup>+</sup> ions intracellularly and K<sup>+</sup> extracellularly in what known as potassium efflux (Nahed et al., 2016; Donia et al., 2018). No doubt, intestinal villi damage and subordinate decreased intestinal absorptive surface are extra causes for the decreased cholesterol (total, HDL, LDL), minerals, electrolytes and trace elements levels in EG (Kumar et al., 2015; Hassanein et al., 2017).

It worth to mention that, the hyperalbuminemia and hyperglycemia stated in EG in relation to CG, PG, AG in this study as well as the increased TLC, lymphocytes, total protein, albumin, globulin, A/G ratio, total lipids, triglycerides, cholesterol (total, HDL), Mg, P, Cl, Na, Cu, Zn, TAC, kidney function tests, liver enzymatic activity, MMP-2 and MMP-9 in EG compared to PG and/or AG are not true and were mostly connected to the hypovolemia and fluid lose due to diarrhea (Zein-Eldin et al., 2013). Logically, the prior anorexia and hypoglycemia resulted in enhancing of the fat stores lipolysis to get energy leading to a massive triglycerides liberation in the diseased animals' circulation. Therefore, a prominent

hypertriglycerdemia and consequent hyperlipidemia were obtained in PG, AG and EG in this work as well as previous studies (Joshi et al., 2015; Bozukluhan et al., 2017; Rodríguez-Carrio et al., 2017).

**Table 4:** *The correlation between MMP-2, MMP-9 and the other clinicopathological parameters in PG, AG and EG (Pearson's correlation test, values = r).*

	MMP-2			MMP-9		
	PG	AG	EG	PG	AG	EG
RBCs	-0.120	-0.002	-0.077	-0.017	-0.179	-0.052
Hb	-0.182	-0.269	-0.137	-0.287	-0.106	-0.042
PCV	-0.070	-0.038	-0.229	-0.097	-0.018	-0.055
MCV	-0.131	-0.007	-0.222	-0.003	-0.178	-0.077
MCH	-0.074	-0.073	-0.030	-0.046	-0.168	-0.033
MCHC	-0.189	-0.191	-0.282	-0.176	-0.073	-0.077
TLC	0.130	0.377	0.324	0.113	0.190	0.545*
Neutrophils	0.034	0.175	0.704*	0.098	0.142	0.030
Lymphocytes	-0.106	-0.278	-0.249	-0.027	-0.060	-0.215
TP	0.421*	0.364	0.043	0.273	0.322	0.073
Albumin	-0.126	-0.101	-0.039	-0.073	-0.124	-0.433
Globulin	0.279	0.073	0.003	0.173	0.028	0.328
ANG	-0.190	-0.042	-0.012	-0.115	-0.084	-0.397
Glucose	-0.078	-0.184	0.460*	-0.066	-0.164	0.513*
Triglycerides	0.66	0.034	0.072	0.249	0.189	0.400
Cholesterol	-0.090	-0.127	-0.040	-0.124	-0.081	-0.023
HDL-cholesterol	-0.090	-0.127	-0.182	-0.124	-0.081	-0.159
LDL-cholesterol	-0.090	-0.127	-0.099	-0.124	-0.081	-0.023
Ca	-0.163	-0.324	-0.008	-0.015	-0.019	-0.271
P	-0.129	-0.032	-0.078	-0.030	-0.001	-0.061
Cl	-0.003	-0.124	-0.312	-0.091	-0.154	-0.076
Na	-0.082	-0.084	-0.460*	-0.095	-0.186	-0.513*
K	-0.071	-0.189	-0.068	-0.071	-0.241	-0.082
Mg	-0.212	-0.213	-0.357	-0.036	-0.033	-0.513*
Cu	-0.036	-0.189	-0.103	-0.028	-0.044	-0.983*
Zn	-0.103	-0.087	-0.114	-0.028	-0.074	-0.975*
TAC	-0.021	-0.123	-0.108	-0.154	-0.195	-0.001
AST	0.127	0.047	0.280	0.127	0.203	0.597*
ALT	0.031	0.118	0.135	0.062	0.180	0.359
ALP	0.259	0.063	0.145	0.002	0.045	0.063
BUN	0.126	0.227	0.263	0.177	0.117	0.186
Cr	0.234	0.365	0.325	0.117	0.063	0.045

Statistical significance of correlations \* was recorded at ( $P < 0.05$ ).

The distinguished decline in the TAC in the three diseased groups was attributed to the aforementioned pro-inflammatory cytokines activity which promotes different immune cells to produce free radicals in large amounts. Fundamentally, these free radicals damage the invading pathogens through reacting with their proteins, DNA and carbohydrates. They are physiologically neutralized by different antioxidants but in the current research they exceed the neutralizing capacity of the antioxidants causing the noticed decreased TAC in PG, AG and EG (Zein-Eldin et al., 2013; Donia et al., 2014; Rodríguez-Carrio et al., 2017). Additionally, the above-mentioned hypo zincemia and hypocupremia in the diseased groups hinder the enzymatic antioxidants synthesis, leading to oxidative stress augmentation as well as lowering of TAC in the diseased animals. Moreover, these free radicals attack different body cells particularly liver and kidney cells result in the observed increase in hepatic enzymatic activities and renal function tests in the diseased groups (Zein-Eldin et al., 2013; Donia et al., 2014; Jesse et al., 2017). The infection metastasis as well as the invading pathogens migration also may take a part in this elevation of the organs function tests (Zein-Eldin et al., 2013; Donia et al., 2014; Jesse et al., 2017).

MMPs are a group of 28 proteolytic enzymes, among them MMP-2 and MMP-9 are the most important (Bircan et al., 2015). Both of them displayed a pronounced activity in sheep inflammatory diseases in this research. Similar results were reported before in human respiratory diseases (Bircan et al., 2015; Li et al., 2016), arthritis (Meszaros and Malemud, 2012) and enteritis (Strup-Perrot et al., 2004; Gao et al., 2005; Garg et al., 2006). Whereas, the pro-inflammatory cytokines (TNF- $\alpha$ , IL-1 $\beta$ ) induce their secretion from various immune cells in order to maintain the immune response by activation of specific proteins, neutrophil migration, reactive oxygen species generation and bacterial phagocytosis (Garg et al., 2006; Meszaros and Malemud, 2012; Li et al., 2016). Additionally, their sensitivity, specificity, LR, PPV, NPV, accuracy rate, percentage of increase and their correlation with some parameters in the diseased groups (PG, EG) in the current work as well as their correlation with the inflammation severity in previous researches confirmed their value as good indicators for sheep pneumonia, arthritis and enteritis and suggested them as new therapeutic target in these conditions (Meszaros and Malemud, 2012; Li et al., 2016).

## Conclusion and Recommendations

Overall then, this research cleared that the ovine pneumonia, arthritis and enteritis are associated with dramatic haemato-biochemical alterations. Following these alterations during the diseases course and counteracting them by supportive treatment may be valuable in reducing the diseases side effects, shortening their duration and enhancing their recovery rates. Both of MMP-2 and MMP-9 is a good inflammatory marker for ovine pneumonia, arthritis and enteritis.

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## Authors Contribution

All authors equally contributed to the work.

## Conflict of interest statement

The authors declare that they have no conflict of interest.

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