CLINICOPATHOLOGICAL STUDIES ON DIAGNOSIS OF LISTERIOSIS IN OSSIMI SHEEP

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ABSTRACT

Between (November 2008-March 2009) a number of 48 Ossimi sheep age (6-12 months) have nervous manifestation and investigated for suspect listeriosis. The diseases sheep were treated with ampicillin and blood sample were collected from all diseases sheep before start the treatment. Diagnosis was achieved by physical examinations, clinical findings, isolation and identification of microorganism, laboratory investigations The selected sheep were categorized into survived group and non survived group in addition to the control group.

Oxidative stress and antioxidant parameters, our results show that SOD, and NO were significant elevated in both responsive and non responsive treatment groups while MDA is significant increased only in non responsive treatment group in compare with control group. Total protein, CK, uric acid, urea and creatinine result show significant elevated in blood level in non-survived group in compare with control one. Regarding to the leukogram there is leukocytosis, neutrophilia in responsive treatment group and lymphopenia in non-responsive group.

In conclusion the oxidative stress, and antioxidant blood parameters are valuable in prognosis the listeriosis in Ossimi sheep.

INTRODUCTION

Listeria monocytogenes is a Gram-positive pathogenic bacterium facultative intracytosolic that has adapted to various environments, from soils and food products to the intestinal tract and intracellular compartments of diverse animal species and humans. Nearly all the domestic animals are susceptible to Listeria infections, but animal listeriosis most commonly occurs in ruminants **(Cooper and Walker, 1998)**. The main clinical features of ruminant listeriosis are encephalitis, septicemia, abortion and mastitis **(Low and Donachie, 1997)**.

The prevalence of encephalitic listeriosis was unexpectedly high when compared to notified confirmed cases in small ruminants **(Oevermann et al., 2008)**.

Haemato logical analysis in ovine listeriosis have a little diagnostic value as leukocytosis is not a consistent feature of listeriosis but only indicative of the possibility of infection

(Bruge're-Picoux, 2008). serum analysis from sheep with ovine meningo-encephalitis showed a significant elevation in the level of creatine kinase and aspartate aminotransferase and a significant reduction in blood bicarbonate, potassium, total plasma protein, albumin and glucose levels **(El-Sawalhy et al., 1999)** meanwhile, in another study, the biochemical finding in sheep and goats with listeriosis revealed a high concentration of total protein, bilirubin, urea nitrogen and the animals had a metabolic acidosis **(Braun et al., 2002)**. Ampicillin and gentamicin have been reported as the treatment of choice for listeriosis **(Bruge're-Picoux, 2008)** .

Antioxidants, such as glutathione, arginine, citrulline, taurine, creatine, selenium, zinc, vitamin E, vitamin C, vitamin A and tea polyphenols help to regulate the ROS thus generated. Antioxidant is further supported with antioxidant enzymes, e.g. superoxide dismutase, catalase, glutathione reductase and glutathione peroxidase those exert synergistic actions in removing free radicals Imbalance between oxidants (free radicals) and reductants (antioxidants) at the cellular or individual level commonly referred as oxidative stress **(Yun-Zhong et al., 2002)**. Unfortunately the immunomodulatory of listeriosis not has been investigated in sheep as human and other domestic animals. Therefore the goal of our study to comparative hematological and biochemical changes associated with survival and non survival listeriosis in sheep.

MATERIALS AND METHODS

2.1. Clinical Examination:

A number of 48 Ossimi sheep in Mansoura Govemorate were examined for suspect listeriosis. The history of disease was variable from mild to severe and characterized by general weakness, respiratory distress, circling movements with twist in the neck beside unilateral facial paralysis developed. Death within one week of the onset of clinical signs of some cases was recorded. The diseases sheep were treated with ampicillin 20 mg//Kg bw for one week and blood sample were collected from all diseases sheep before start the treatment. A selection total of eight native breed Ossimi sheep of both sexes aged between (6-12 months) were studied after diagnosed of listeriosis. Diagnosis of such clinical condition was achieved by competent case history, thorough physical examinations, clinical findings, isolation and identification of microorganism, laboratory investigations. In addition to twenty apparently healthy Ossimi sheep of both sexes aged between (6- 12 months), were randomly selected and were considered as a control group. The selected sheep were categorized into survived group and non survived group in addition to the control group. (Each group is eight, four females and four males).

2.2 Bacteriological investigations:

Samples for bacteriological examination were collected from cerebrospinal fluid and tissues from hind brain (medulla oblongata, and anterior part of spinal cord) liver, lung, kidney and spleen from all diseases sheep (recent mortality sheep as well as which were sacrificed at the terminal stages on 6th day post clinical symptoms).

Samples were inoculated directly onto blood agar, MacConkey's and McBride's medium. These samples were processed as per standard protocol of **Gray and Killinger**

(1966) and various biochemical tests (motility at 25°C, aesculin hydrolysis, fermentation of alpha methyl d-mannose, mannitol, ribose, rhamnose and xylose, nitrate reduction, methyl red and voges proskauer) for characterization of L. monocytogenes were performed using a commercial system according to Seeliger and Jones(1986).

2.3. Blood Samples:

Blood from the affected and healthy sheep was collected for haemato logical, biochemical and immunological examination. Two types of venous blood samples (five ml for each) were collected via jugular vein puncture from each sheep; the first blood samples added to 5mg sodium ethylene diamine tetra acetic acid (EDTA) as anticoagulant for hematological evaluation of total erythrocytic cell counts, packed cell volume (PCV %), hemoglobin total and differential leucocytic count according to the method described by **Coles (1986)**; whereas the second blood samples were collected into heparenized syringe to collect blood plasma which was separated quickly and kept frozen for biochemical analysis of aspertate amino transferase (AST), ALT, total bilirubin and, total protein, albumin, glucose, urea , creatinine and uric acid (UA) and superoxide dismutase (SOD), reduced glutathione (GSH) malondialdehyde (MDA), nitric oxide, nitric oxide (Biodiagnostic Egypt).

2.5. Statistical analysis:

Data was subjected to statistically analyzed by ANOVA test with posthock LSD multiple comparison test using statistical software program (SPSS for windows version. 15, USA). Differences were considered significant at $P < 0.05$.

RESULTS & DISCUSSION

From total examined (48) diseases sheep samples from brain tissues and CSF, a (21) isolate was recovered. The isolates were grayish white small dew drop like colonies observed on blood agar with narrow zone of p-haemolysis. Growth was attained on MacConkey's agar and on McBride's selective medium after direct inoculation the organisms were present parallel to each other giving stake appearance in groups of two or three or scattered singly with gram stain smear. Some organisms had attained the vertical position giving dot like or cocci appearance. Confirmatory test results using the some commercial chemical tests, the organism showed tumbling motility at 25 & deg C, positive reaction for methyl red aesculin hydrolysis, fermentation of alpha methyl d-mannose and rhamnose, MR and voges proskauer. Biochemical tests were negative for nitrate, urease, indole reduction and fermentation of mannitol, ribose and xylose. No other significant pathogens were recovered from the specimens. Also no significant pathogens were recovered from control groups. The epidemiologic screening of ovine flocks could be a helpful preventive measure, especially if rapid and sensitive diagnostic procedures are employed and could represent an effective approach to the epidemiological screening of ovine flocks.

Diagnosis of animal listeric infection is currently achieved by microbiological or histological tests.

Increase incidence of listeriosis were recorded in the winter season **(November-March 2008)** could attributed to at this period weather conditions are favorable for the growth of bacteria due to very low environ-

mental temperature and the ability of the L. monocytogenes to grow very well at reduced temperatures compared to other mesophilic medically important microorganisms **(Paul et al., 2006 & Kumar et al., 2007)**. Our results partial agree with **Al-Dughaym, et al., (2001)** who recovery L. monocytogenes from brain and some cases from lung in outbreak septicaemic listeriosis in sheep.

Lipid peroxidation is known to have a role in many infectious diseases. The mechanism of damage involves lipid peroxidation, which destroys cell membranes with' the release of intracellular components, such as lysosomal enzymes, leading to further tissue damage **(Demir et al., 2003)**. Our results show that SOD, and NO were significant elevated in in both responsive and non responsive treatment groups while MDA is significant increased inly in non responsive treatment group in compare with control group. Malondialdehyde (MDA) is a by-product of lipid peroxidation and used as an index ofoxidative stress in cells and tissues **(Cevat et al., 2007)**. Increase oxidative stress parameters in our work documented by elevation of MAD, SOD and uric acid and decrease antioxidant parameters in non-survived infected group. **Leib and Tauber (1999) & Aycicek et al., (2006)**, reported increase oxidative stress and decrease antioxidant blood parameters in patients suffer from bacterial meningitis.

Ah met and **Ay sen (2007) & Fry et al., (1993)**, concluded the bacterial infection in sheep induces lipid peroxidation, leading to a rapid consumption of the antioxidant from the body.

The liver enzyme in our study is insignificantly elevated in non-survived group in compare with both survived and control groups, Shaw, (2008). Zundel and Bernard (2006) studied experimental infection of sheep with L monocytogenes and observed the all organs of gastrointestinal tract were infected included liver and spleen. Hepatopathy and hepatic necrosis as a result of listerosis were recorded in sheep, lamb, calves, llama and guinea pigs **(dark, et al., 2004, Burdarov and Savova-Burdarova, 1987, Seimiya et al., 1992 Semrad, 1994 & Elizabeth et al., 2008)** respectively.

Hypoalbuminemia results from a derangement in one or more of these processes **(Don and Kaysen 2004)**. Hypoalbuminemia in non-survived sheep could be attributed to malnutrition and or liver dysfunction.

Glucose is non significant in both survived and non-survived group in compare with control group. Sepsis, which causes inadequate glucose to be delivered to the body's cells, may also cause hypoglycemia **(Thompson 2008)**. Also hypoglycemia recorded associated with bacterial infection in lamb **(Burkhard and Garry 2004)**.

Creatine kinase isoenzymes are characteristic for skeletal muscle, myocardium, and brain **(Grzyb and Skorkowski 2008)**. The elevation this enzyme in our work could be as a result of brain damage caused by listeriosis. Lesion damage associated with listeriosis in sheep has been reported by **Brug'ere-Picoux (2008), Kumar et al., (2007) and Al-Dughaym, et al., (2001)**.

Regarding to kidney function test our result show significant elevated in uric acid, urea and creatinine blood levels in non-survived group. **Low and Donachie (1991), Low and Renton (1985), Evans, and Watson (1987)**, reported the septicemic listeriosis lesions were severe in brain, liver, spleen and lymph nodes in lamb, sheep and calf respectively & **Kumar et al., 2007)**. Our results partial agree with **Al-Dughaym, et al., (2001)** who recovery L. monocytogenes from brain and some cases from lung in outbreak septicaemic listeriosis in sheep.

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Regarding to hematological data of our work is show leukocytosis, neutrophilia in responsive treatment group and lymphopenia in non-responsive group.

Wood (1972) reported anemia, leukocytosis, neutrophilia with lymphopenia in goats suffer from encephalitic listeriosis, this finding in line with our hematological results. In the same aspect **Joze et al., (2008)** observed anemia, neutrophilia and lymphopenia in cattle infected with L. monocytogenes. **Brug'ere-Picoux (2008)** concluded that Leucocytosis is not a consistent feature of the listeriosis in sheep and only indicates the possibility of an infection.

CONCLUSION

We concluded that blood oxidative stress, antioxidant and leukogram parameters are valuable in prognosis the listeriosis in Ossimi sheep.

Table (1) : Hematological values (mean values & plusmn SE) in Clinically Healthy Sheep, Responsive and Non-Responsive Listerial Cases to Treatment.

Groups	TLC $10^3/\mu L$	Lymph $10^3/\mu L$	Neutro $10^3/\mu L$	Mono $10^3/\mu L$	Eosino $10^3/\mu L$	Baso $10^3/\mu L$	Band $10^3/\mu L$
Cont	14.81^{ab}	7.25^{a}	6.19^{a}	0.40^{a}	0.40^{a}	0.00^{a}	0.57^{a}
(n=8)	±0.58	±0.53	±0.40	±0.15	±0.17	±0.00	±0.26
Response	16.93^{b}	5.57^{ab}	9.62^{b}	0.38^{a}	0.67^{a}	0.00^{a}	0.49^{ab}
(n=8)	±0.45	±0.72	±0.71	±0.20	±0.10	±0.00	±0.15
No Response	13.22^{a}	4.20^{b}	7.20^{a}	0.20^{a}	0.60^{a}	18.75^{a}	0.24^{b}
(n=8)	±0.38	±0.86	±0.88	±0.16	±0.09	±18.75	±0.00

Table (2) : Different Serum Antioxidant Levels and Other Oxidative Stress Markers (mean values & plusmn SE) in Clinically Healthy Sheep, Responsive and Non- Responsive Listerial Cases to Treatment.

Groups	MDA nmol/ml	SOD U/ml	GSH mg/dl	Catalase U/L	No µmol/l
Cont	5.46^{a}	163.84^{a}	1.35^{a}	332.36^{a}	16.05^{a}
(n=8)	±0.33	±3.19	±0.16	±11.36	±0.41
Response	8.26^{a}	213.12^{b}	1.25^{a}	378.19^{a}	20.50^{b}
(n=8)	±0.93	±9.27	±0.16	±39.34	±0.61
No Response	13.87^{b}	213.00^{b}	1.16^{a}	383.50^{a}	18.43^{c}
(n=8)	±1.42	±9.30	±0.12	±41.66	±0.53

Table (3) : Serum Biochemical values (mean values ± SE) in Clinically Healthy Sheep, Responsive and Non- Responsive Listerial Cases to Treatment.

Groups	ALT U/L	AST U/L	CK U/L	Creatinine mg/dl	U. A mg/dl	Urea mg/dl
Cont	60.79^{a}	202.21^{a}	96.00^{a}	0.85^{a}	1.58^{a}	47.00^{a}
(n=8)	±0.59	±5.18	±5.24	±0.02	±0.16	+2.44
Response	57.62^{a}	196.72^{a}	141.25^{a}	0.90^{a}	1.68^{a}	49.25^{a}
(n=8)	±0.41	±6.53	±12.35	±0.04	±0.06	+2.78
No Response	65.04^{b}	209.87^{a}	410.50^{b}	1.19^{b}	2.54^{b}	58.88^{b}
(n=8)	±2.39	±4.52	±46.71	±0.03	±0.13	±2.77

Groups	T. P g/dl	Album g/dl	Blobulin g/dl	A / G Ratio	Glucose mg/dl
Cont	8.55^{ab}	4.13^{a}	4.42^{a}	0.95^{a}	70.75^{a}
(n=8)	±0.23	±0.15	±0.19	±0.06	±3.88
Response	7.82^{a}	3.23^{b}	4.60^{ab}	0.74^{a}	71.00^{a}
(n=8)	±0.36	±0.21	±0.36	±0.09	±6.66
No Response	9.87^{b}	3.93^{a}	5.94^{b}	0.72^{a}	73.50^{a}
(n=8)	±0.87	±0.31	±0.70	±0.08	±6.33

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الملخص العربى

دراسات باثولوچيا إكلينيكية على تشخيص مرض الليستريا فى الأغنام الأوسيمى

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قسم الباثولوچيا الإكلينيكية - كلية الطب البيطرى - جامعة المنصورة

المنصورة - 35516 - مصر

أجريت هذه الدراسة على عدد (48) من الأغنام الأوسيمى تتراوح أعمارها من (6-12) شهر والتى تعانى من وجود أعراض عصبية وذلك خلال الفترة الزمنية من نوفمبر 2008 إلى مارس 2009 ، تم علاج الأغنام المصابة بالأمبسيللين وتجميع عينات الدم من كل الأغنام المصابة قبل بداية العلاج، تبين من خلال الفحص الإكلينيكى، الأعراض الظاهرة على الحيوانات، عزل وتصنيف الميكروب فى (21) حالة والتشخيص المعملى أن هذه الأغنام مصابة بمرض الليستريا ، وقد تم تقسيم الأغنام المصابة إلى مجموعتين : الأولى إستجابت للعلاج والثانية لم تستجيب للعلاج ومجموعة من الأغنام السليمة كمجموعة ضابطة.

دلائل الأجسام المؤكسدة ومضادات الأكسدة أوضحت وجود زيادة معنوية فى إنزيم السوبر أوكسيد ديسميوتيزل وأول أوكسيد النيتروچين فى المجموعتين مقارنة بالمجموعة الضابطة، على الرغم من الزيادة المعنوية لإنزيم المالونداياالدهيد فى المجموعة التى لم تستجيب للعلاج فقط مقارنة بالمجموعة الضابطة، أيضاً أظهرت قياسات البروتين والكريتين كيناز واليوريك أسيد واليوريا والكرياتينين وجود زيادة معنوية مقارنة بالمجموعة الضابطة، وقد أوضحت صورة الدم وجود زيادة فى عدد كرات الدم البيضاء وخاصة الخلايا المتعادلة فى المجموعة المستجيبة للعلاج مع النقص فى الخلايا الليمفاوية فى المجموعة التى لم تستجيب للعلاج.

خلصت هذه الدراسة إلى أن قياس مضادات الأكسدة والمؤشرات الدالة على وجود الأجسام المؤكسدة ذو أهمية كبيرة فى التنبوء بمدى إستجابة الأغنام المصابة بمرض الليستريا.