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STUDIES ON SOME BACTERIAL CAUSES AND BLOOD SERUM BIOCHEMICAL CHANGES OF RESPIRATORY AFFECTIONS IN LAMBS

(With 6 Tables)

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دراسات عن بعض المسببات البكتيرية والتغيرات البيوكيميائية لمصل الدم فى الإصابات التنفسية للحملان

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أجريت هذه الدراسة علي عدد ٣٦ من الحملان في إحدى المزارع الخاصة بمحافظة الدقهلية منها ٧ من الحملان السليمة ظاهريا ، ١١ من الحملان المريضة إكلينيكيا و١٨ من الحملان المذبوحة. وقد تم أخذ عينات للفحص البكتريولوجي وكذلك عينات دم وسيرم للفحوصات الدموية والبيوكيميائية وبالفحص البكتريولوجي للعينات تبين أن ٣٥ عينة إيجابية للعزل البكتيري بنسبة ٦ (٨٥,١٧%) ،١١(١٠٠%) و١٨(١٠٠%) من السليم ظاهريا، والمريض إكلينيكيا والحملان المذبوحة علي التوالي. وتم عزل ٦٦ معزولة بكتيرية صنفت بيوكيميائيا إلي باستريلا مالتوسيدا ١٩(٢٨,٧٩%) ، باستريلا هيموليتيكا ٥ (٧,٥٨ %) ، الميكروب القولوني ١٥ (٢٢,٧٣%) ، كليبسيلا نيمونيى٨ (١٢,١٢%) ، سودوموناس إرجينوزا ٧(١٠,٦١%) والميكروب الذهبي العنقودي ١٢ (١٨,١٨ %). وبإجراء اختبار ضراوة معزولات الباستريلا ملتوسيدا كانت كل المعزولات من النوع الممرض. وبإجراء الفحوصات الدموية والبيوكيميائية لعينات الدم المأخوذة من الحيوانات السليمة ظاهريا والمريضة إكلينيكيا تبين أن التغيرات في صورة ومصل الدم في الحملان المصابة باضطرابات تنفسية عند مقارنتها بالسليم ظاهريا هي: ١- وجود نقص معنوي عالي في العد الكلي لكرات الدم الحمراء وتركيز الهيموجلوبين ، حجم الخلايا المضغوطة وخلايا الليمفوسيت وفي الجانب الآخر وجدت زيادة معنوية عالية في العد الكلي لكرات الدم البيضاء ومعنوية للخلايا المتعادلة والحمضية وخلايا المونوسيت. ٢- وجود زيادة معنوية عالية في مستوى كل من أنزيمات الترانس أمينيز بالكبد (S.GGT, S.ALT, S.AST) وكذلك بولينا الدم وزيادة معنوية في مستوى الكرياتينين. ٣- وجود نقص معنوي عالٍ في مستوي الالبيومين ونقص معنوي في مستوي كل من البروتين الكلي، والصوديوم ، الكلورايد والكالسيوم. وعلي النقيض وجدت زيادة معنوية في مستوى سكر الدم والبوتاسيوم. مما سبق يتضح أن الإصابات التنفسية في الحملان ذات الأصل البكتيري تسبب تغيرات معنوية في صورة الدم

وفي وظائف الكبد والكلى وكذلك تؤثر علي مستوى كل من عناصر الصوديوم ، البوتاسيوم، الكلورايد والكالسيوم.

SUMMARY

This study was carried out on 36 lambs in private farm at Dakahlia governorate, 7 of them apparently healthy, 11 clinically diseased suffering from respiratory affections and 18 slaughtered lambs. Bacteriological examination of the samples revealed that 35 samples were positive for bacterial isolates, distributed as 6 (85.71%), 11 (100.00%) and 18 (100.00%) of apparently healthy, clinically diseased and slaughtered lambs respectively. 66 bacterial isolates identified biochemically into P. multocida 19 (28.79%), P. heamolytica 5 (7.58%), E. coli 15 (22.73%), Klebsiella pneumonia 8 (12.12%), Pseudomonas aeruginosa 7 (10.61 %) and staph. aureus 12 (18.18%). Pathogenicity test for P. multocida isolates indicated that all isolates were pathogenic. Blood samples were collected from the clinically healthy and diseased groups of lambs. Two blood samples were obtained from each animal, one as a whole blood and the other in the form of blood for obtaining clear non-haemolysed serum. Haematological studies revealed that presence of high significant decrease in the total erythrocytic count, haemoglobin concentration, packed cell volume and blood lymphocytes (per mm^3 blood) in diseased animals when compared with clinically healthy ones. Also high significant increase in total leucocytic count, significant increase in neutrophil, oesinophil and monocyte cells were recorded in diseased cases.The studied biochemical parameters revealed high significant elevation in the values of AST, ALT, GGT and blood urea in diseased animals. Also creatinine showed marked elevation. Presence of high significant decrease in the level of albumin, while total protein, sodium chlorides and calcium levels were significantly decreased. On contrary, presence of significant increase in the glucose and potassium levels. From previously mentioned data, it was cleared that respiratory affections in lambs especially those of bacterial origin cause significant changes in blood picture, liver and kidney functions and the level of both sodium, potassium, chloride and calcium.

Key words: *Bacterial causes, Blood serum, Respiratory affection, Lambs.*

INTRODUCTION

Respiratory affections constitute a common problem in sheep, particulary lambs causing serious economic losses and mortalities.

Wilson *et al.*, (1985) and Radostits *et al.*, (2002). The causes of respiratory affections in lambs are bacteria, viruses, fungi, parasitesand and other bacteria are the main cause of respiratory affections of sheep Soroor (1999). While poor hygienic measures and climatic disorders were the most predisposing factors to such infection Sharma and Woldeh, (1995). Respiratory troubles to all domestic animals were attributed to mixed infection with different bacteria isolates. (Yehia, 2000 and Wafaa, 2003). Pasteurella spp., klebsiella spp., E. coli, Pseudomonas spp. and staphylococcus aureus are the most bacterial causes of. Lambs pneumonia (Martin 1996 and Bedier 2003). Pasteurella spp. were the main causative agents of the respiratory affection Sadiek *et al.*, (1993). The inflammatory lung diseases were generally accompanied by noticable drop of erthrocytic counts and marked elevation of total leucocytic counts, further more in advanced cases hepatic and renal dysfunctions were noticed (Soroor, 1999 and Rodostitis *et al.*, 2002).

The aim of the present work was directed to study :–

1- Isolation and identification of bacterial causes of respiratory affections in lambs.
2- Pathogenicity of the isolates to laboratory animals.
3- Some haematological and biochemical changes associated with respiratory affections to aid in early diagnosis of such affections.

MATERIALS and METHODS

Animals:

The present study was carried out on 36 lambs aged from 12- 18 months located at a private farm in Dakahlia Governorate. The animals were fed on barseem and dry ration. Eighteen of these lambs were suffering from respiratory disorders were slaughtered. The rest were divided into two groups, 7 clinically healthy and 11 diseased lambs showing signs of respiratory troubles including rapid breathing, moist rales, congested mucous membranes, mucopurulent nasal discharge, severe dysponea and pyrexia in (Rectal temperature 40 – 41°C). Parasitological examination was done for the diseased lambs to exclude those infested with internal parasites.

Samples:

Bacteriological samples:

Nasal swabs were collected from all animal groups. The tracheal swabs and lung tissues were only collected from the slaughtered lambs.

The samples were taken under aseptic conditions and sent without delay to the laboratory for bacteriological examinations as follow:

Nasal and tracheal swabs were inoculated into nutrient broth and incubated at 37°C for 24 hr. and then subcultured into 5% blood sheep agar, MacConkeys agar and nutrient agar and incubated at 37°C for 24-48 hr. The surface of the lung tissues were sterilized with a hot spatula then, the tissue was incisized with sterile scalpel and with sterile platinum loop samples were taken and inoculated in the previously mentioned media. The produced colonies were identified by their morphology and biochemicals activities according to Koneman *et al.*, (1997) and Quinn (2002).

Pathogenicity and virulence of isolated Pasteurella multocida:

This was done according to Wessman (1964) where 51 Swiss Webster white mice of (18 – 22 gram weight) were used. The mice was injected intra peritoneally by 0.1 ml of bacterial suspension (1.5×10^8 organism per ml.). All dead mice showed postmortem changes. Reisolation of inoculated strains from heart blood of dead mice was carried out.The prepared blood films were stained with leshiman's stain for showing the characteristic features of P. multocida organisms.

Blood Samples:

Two blood samples were collected from each lamb via jugular vein puncture. The first samples were whole blood collected in vacutainer tubes containing EDTA as anticoagulant and were used for haematological studies according to Jain (2000). The second samples were collected in centrifuge tubes and allowed to clot at 37°C and then nonhaemlysed blood serum was separated used for measuring total protein according to (Doumas *et al.*, 1981), albumin (Frank, 1950), glucose (Trinder, 1969), blood urea (Coulmobe 1963), creatinine (Husdan and Rapoport 1968), serum AST, ALT (Reitman and Frankel 1957) and Serum GGT (Szasz, 1969). Using commercial diagnostic kits supplied by BioMerieux France, while total globulin calculated mathematically by subtracting albumin from total protein. Blood serum Sodium, potassium and chloride were determined using flame photometer (Oser, 1979). Serum Calcium was determined according to (Gindler and King 1972).

Statistical analysis :-

All Data were subjected to statistical analysis according to Snedcor and Cochran, (1982).

RESULTS

All bacteriological, haematological and serum biochemical results were illustrated in Tables (1 – 6).

Table 1: Incidence of bacteriologically positive cases in examined lambs.

Condition of lambs	Examined samples	Positive samples		Negative samples		Bacterial isolation data 0f examined samples						No. of bacterial isolates.
						Samples with one isolate		Samples with two isolates		Samples with three isolates		
		No.	%	No.	%	No.	%	No.	%	No.	%	
A- living lambs	18	17	94.44	1	5.56	10	58.82	3	17.65	4	23.53	28
1- clinically healthy	7	6	85.71	1	14.29	6	100.00	-	-	-	-	6
2- Diseased	11	11	100.00	-	-	4	36.36	3	27.28	4	36.36	22
B- Diseased slaughtered lambs	18	18	100.00	-	-	5	27.78	6	33.33	7	38.89	38
Total	36	35	97.22	1	2.78	15	42.86	9	25.71	11	31.43	66

Table 2: Type, incidence and frequency of bacteria recovered from examined Lambs.

Organisms	Condition of lambs										Total	
	Living lambs				Diseased slaughtered lambs							
	Clinically healthy		Diseased		Nasal swab		Tracheal swab		Lung tissue			
	No.	% *	No.	% *	No.	% *	No.	% *	No.	% *	No.	% **
Pasteurella multocida	2	5.71	6	17.14	4	11.42	2	5.71	5	14.29	19	28.79
Pasteurella haemolytica	0	0	2	5.71	1	2.86	1	2.86	1	2.86	5	7.58
E. coli	1	2.86	5	14.29	3	8.57	2	5.71	4	11.42	15	22.73
Klebsiella pneumoniae	1	2.86	3	8.57	2	5.71	0	0	2	5.71	8	12.12
pseudomonas aeruginosa	2	5.71	1	2.86	2	5.71	1	2.86	1	2.86	7	10.61
Staph . aureus	0	0	5	14.29	3	8.57	1	2.86	3	8.57	12	18.18
Total	6	17.14	22	62.86	15	42.86	7	20.0	16	45.71	66	100.00

*The percentage was calculated according to the positive number of samples (35)
** The percentage was calculated according to the total number of isolates (66)

Table 3: Pathogenicity of isolated Pasteurella multocida in mice.

No. of isolates	No. of inoculated mice	Time of death			
		Less than 24 hr	24 hours	48 hours	72 hours
19	57	18	27	12	0

Table 4: Mean values of haematological parameters in clinically healthy and respiratory affected lambs.

Item / Parameters	Clinically healthy lambs	Respiratory affected lambs
Total RBCs ($x10^6$ / mm^3)	8.01 ± 0.23	7.13 ± 0.12 **
Hb (g / dl)	11.97 ± 0.22	10.78 ± 0.20 **
PCV (%)	37.12 ± 1.29	32.97 ± 1.39 **
Total WBCs ($x10^3$ / mm^3)	10.83 ± 0.44	12.67 ± 0.37 **
Neutrophils (%)	37.82 ± 0.62	39.92 ± 0.49 *
Eosinophils (%)	2.25 ± 0.33	3.21 ± 0.24 *
Basophils (%)	0.31 ± 0.02	0.29 ± 0.01 N.S
Lymphocytes (%)	54.98 ± 0.96	49.11 ± 1.20 **
Monocytes (%)	4.64 ± 0.59	7.47 ± 1.13 *

* : significant at ($p < 0.05$).
** : highly significant at ($p < 0.01$).
N.S : non significant .

Table 5: Mean values of liver and kidney functions in clinically healthy and respiratory affected lambs.

Item / Parameters	Clinically healthy lambs	Respiratory affected lambs
Serum. AST (IU / l)	38.67 ± 1.04	43.04 ± 0.90 **
Serum. ALT (IU / l)	24.73 ± 0.98	29.86 ± 1.09 **
Serum . GGT (IU / l)	18.51 ± 1.26	24.62 ± 1.19 **
B . urea (mg / dl)	28.15 ± 1.63	36.23 ± 1.37 **
Creatinine (mg / dl)	1.74 ± 0.25	2.45 ± 0.16 *

* : significant at ($p < 0.05$).
** : highly significant at ($p < 0.01$).

Table 6: Mean values of some biochemical parameters in clinically healthy and respiratory affected lambs.

Item / Parameters	Clinically healthy lambs	Respiratory affected lambs
Total protein (G / dl)	7.55 ± 0.38	6.36 ± 0.27 *
Albumin (G / dl)	3.47 ± 0.33	2.16 ± 0.29 **
Globulin (G / dl)	4.08 ± 0.18	4.21 ± 0.20 NS
Glucose (mg / dl)	51.42 ± 1.62	56.93 ± 1.64 *
Sodium (mmol / l)	143.27 ± 2.29	136.08 ± 1.66 *
Potassium(mmol / l)	4.30 ± 0.28	5.61 ± 0.25 **
Chloride (mmol / l)	97.23 ± 1.95	90.60 ± 1.71*
Calcium (mg / dl)	12.06 ± 0.27	10.99 ± 0.25 *

* : significant at ($p < 0.05$).
** : highly significant at ($p < 0.01$).
NS : Non significant.

DISCUSSION

Commensal bacteria present in the respiratory system may cause respiratory disease when animals are subjected to stress factors (Palotay and Newhall 1985). The prominent clinical signs which were observed in this study were similar to those recorded by (Attia and Eassa 1997 and Hatem *et al.,* 2003). Results recorded in Table (1) revealed that the higher incidence of bacteria was obtained from clinically 11 diseased lambs (100%) and (100%) from 18 slaughtered lambs as compared to healthy 6 ones (85. 71%). While Ibrahim and Selim (2003) isolated bacteria from clinically healthy, diseased and dead lambs with an incidence of 20%, 66.67 and 78.26% respectively.

The results of identification of the obtained isolates using biochemical tests revealed that isolation of P. multocida, P. haemolytica, E. coli, klebsiella pneumoniae, Pseudomonas aeruginosa and staph. aureus as shown in (Table 2). These findings agreed with those reported by Yehia (2000) and Bedier (2003).

In our investigation, the bacteriological incidence revealed that isolation of P. multocida. and pseudomonas aeruginosa were 2 (5.71%) for each one and. 1 (2.86%) for each E.coli and Klebsiella pneumoniae from clinically healthy lambs as shown in (Table 2). On theother hand

Elyas (1993) who isolated Staph. aureus (26%), E. coli (16%) and P. multocida (3%) from clinically normal lambs. Bacteriological examination (Table 2) of clinically diseased lambs revealed that the isolation of P. multocida 6 (17.14%), P. haemolytica 2 (5.71%), Klebiella pneumoniae 3 (8.57%), Pseudomonas aeruginosa (2.86 %) and 5 (14.29%) for each E. coli and staph. aureus. These resrlts were nearly similar to those reported by Hatem *et al.,* (2003) who isolated P. multocida, p. haemolytica, E. coli, Klebsiella pneumoniae, Pseudomonas aeruginosa and staph. aureus from diseased sheep with respiratory affections. The data present in (Table 2) showed that the positive bacteriological examination of nasal, tracheal. and lung samples, from slaughtered lambs were 15 (42.86), 7 (20.0%) and 16 (45.71%) respectively. Where the types of isolates were P. multocida, P. haemolytica, E. coli, klebsiella pneumoniae, pseudomonas aeruginosa and staph. aureus. Similar findings were recorded by Elyas (1993) and Hatem *et al.*, (2003).

The pathogenicity of P. multocida isolates to white mice (Table 3) revealed that all isolates were highly pathogenic to mice producing acute septicemia and death within 48 hours post inoculation. This finding agreed with the result obtained by Aliaa (2002).

Dealing with the changes in the haematological parameters in diseased lambs (Table 4), showed microcytic hypochromic anemia represented by high significant reduction ($p< 0.01$) in erythrocytic counts (RBCs), hemoglobin concentration (HB), and packed cell volume (PCV%). These findings could be attributed to the failure of bone marrow cells and hepatocytes in utilization and hemoglobin synthesis resulting in inhibition of erythropoiesis during bacterial infection Kaneko *et al,* (1997). These results agreed with those recorded by El- Bealawy and Marouf (2002) and Radostits *et al.,* (2002).

Table (4) showed high significant increase ($p<0.01$) in total leucocytic count and significant increase ($p<0.05$) in neutrophils, oesinophils and monocytes. On the contrary marked reduction ($p<0.01$) in lymphocytes was observed. These findings were supported by those obtained by Sadiek *et al.,* (1993) who referred such haematological changes to bacterial infection and inflammatory lesions that acts as severe stimuli for production of mature and immature neutrophils.

The obtained data concerning liver functions tests, (Table 5) showed a marked elevation ($p<0.01$) of all measured liver enzymes including Serum AST, Serum AlT and Serum GGT. Haziroglue and Kul (2004) declared that such elevation in liver enzymes referred to the

degeneractive and necrotic changes of the liver following the bacterial infection and its circulating toxins. These results come in accordance with those reported by Attia and Eassa (1997), Kodary and Abdalla, (2001).

The respiratory affected lambs showed high significant and significant increase ($p<0.01$ & $p<0.05$) in serum urea and cretinine levels respectively (Table 5). These findings fitted closely with those of Abdalla and Emam, (2005). Radostitis *et al.,* (2002) referred the increases of blood. urea and certinine values to the increase in protein catabolism, febrile conditions disease, causing impaired cardiac function and decreased renal blood flow as well as renal damage from bacterial effects.

Significant drop were observed in both blood serum, total protein and albumin (Table 6). Omran *et al.,* (2005) said that the hypoproteinaemia and hypoalbuminaemia could be due to the state of anorexia and inability of the liver to synthesise protein. The result of protein pattern in the current work agreed with those recorded by El-Seidy *et al.,* (2003) and Hatem *et al.,* (2003).

The results of the respiratory affected lambs revealed significant increase ($p<0.05$) in glucose levels. These findings coincide with those obtained by Abdalla and Emam (2005). Coles (1986) attributed the hyperglycemia to anorexia on one hane and to liver glycogen unstability in deficient oxygen supply in diseased lambs on the other side.

Concerning serum electrolytes, (Table 6) showed significant hyponatraemia, hypochloremia ($p<0.05$) and hyperkalamia ($p<0.01$) in diseased lambs. The recorded changes might be attributed to the hyperpyrexia in the acute course of the disease and metastatic infection of liver and kidneys resulting in hepatic and renal dysfunction (Soroor 1999) and Novert (2004). Similar results were recorded by Omran *et al.,* (2005). Radostits *et al.,* (2002) attributed the hypocalcemia to the anorexic state and intestinal malabsorption.

Finally, it could be concluded that the recovered pathogenic and potentially pathogenic isolates have an important role in the respiratory affection, So adequate hygienic measures and proper management may reduce the degree of animals exposure to disease producing agents. Respiratory affections specially those of bacterial origin were accompanied with some reversible adverse effects on animal health represented by hepatic, renal dysfunction and disturbance of haematological patterns, so we can put the previously concluded points in our consideration in diagnosis and treatment respiratory affections.

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