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STUDIES AFFECT CASES OF BACTERIAL RESPIRATORY INFECTION ON SOME BIOCHEMICAL CHANGES IN CAMELS AT ASSIUT GOVERNORATE

(With 5 Tables)

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دراسة تأثير الإصابات البكتريولوجية للجهاز التنفسى على بعض التغيرات البيوكيميائية فى الإبل بمحافظة أسيوط

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أجريت هذه الدراسة على عدد 90 جملاً مذبوحين من الجنسين ومن مختلف الأعمار فى سلخانة بنى عدى بمحافظة أسيوط ، أظهر الفحص الإكلينيكى قبل الذبح بأن 25 من الجمال كانت تعانى من إصابات تنفسية متمثلة فى صعوبة فى التنفس مصحوبة بكحة وإفرازات مخاطية وباقى الجمال سليمة من الناحية الإكلينيكية الظاهرية. اشتملت الدراسة على 100 عينة (25 مسحة من الأنف ، 25 عينة من كل من الرئة والغدد الليمفاوية الرئوية والسائل البلورى) وذلك لفحصها بكتريولوجياً من الحيوانات المريضة إكلينيكياً. أما الحيوانات السليمة ظاهرياً أخذت منها مسحات من الأنف (65) قبل الذبح لفحصها بكتريولوجياً. أعطت 103 (62.42%) عينة نتائج إيجابية للعزل الميكروبى ومن بينهم 20 (30.76%) من المسحات الأنفية للحيوانات السليمة ظاهرياً. وكانت الميكروبات المعزولة كالأتى: العنقودى الذهبى 47(45.63%) ، السبحى الصديدى 17 (16.50%) والكلبسيلا 2 (1.94%) واستافيلوكوكس إبيديرميس 17 (16.50%). كما تم تصنيف الميكروب القولونى سيرولوجياً إلى 15 (14.55%) E.Coli (O_{126}/B_6) ، 5 (4.35%) لم يصنفا. وبإجراء الفحوصات البيوكيميائية لعينات مصل الدم المأخوذة من الحيوانات السليمة ظاهرياً والمريضة إكلينيكياً قبل الذبح تبين وجود انخفاض معنوى جداً في نسبة الحديد مع ارتفاع معنوى جداً فى تركيز النحاس في الجمال المريضة بالمقارنة مع السليمة. وقد شملت الدراسة أيضاً معرفة مدى تأثير الإصابات البكتيرية الرئوية على غازات الدم والاتزان الحمضى القاعدى وقد أظهرت النتائج وجود نقص معنوى جداً فى قيم الأس الهيدروجينى وانخفاض معنوي في قيم كل من B.E & Po_2 بينما لوحظ ارتفاع معنوى فى قيم PCo_2 فى الجمال المريضة عند مقارنتها بالمجموعة السليمة. وتم عمل اختبار حساسية للميكروبات المعزولة ووجد أن الجاراميسين هو أنسب المضادات الحيوية معملياً ضد البكتريا المعزولة وهو الأكثر تأثيراً عليها.

SUMMARY

This study was carried out on ninety slaughtered camels of different ages and sex in Bani-Adi abattoir at Assiut Goverenorate. The clinical examination before slaughter proved that 25 camels had respiratory affections (Dyspnoea, cough, harried respiration and mucoid nasal discharge) and the remaining were apparently healthy clinically served as control group. 100 samples (25 nasal swabs and 25 from each of lung, pulmonary lymph nodes and pleural fluid) were tested for bacteriological examination from diseased \camels.Also in clinically healthy camels 65 nasal swabs were taken before slaughter for bacteriological examination. 103 (62.42%) samples were culturally positive for different microorganisms, from which 20 (30.76%) nasal swabs from clinically healthy camel. Bacteriological examination revealed that the main isolates were Staph. aureus 47 (45.63%), Strept. pyogenes 17 (16.50%), Klebsiella species 2 (71.97%) and Staph. epidermidis 17 (16.50%). The isolated *E.Coli* strains were identified serologically into 15 (14.55%) *E.coli* strain (O_{126}/B_6) and 5 (4.35%) untypable. Blood samples from clinically healthy and diseased animals were taken before slaughter for biochemical analysis. It showed that highly significant ($P < 0.01$) decrease in iron level, while there was a highly significant increase in copper levels in case of clinically diseased camels in comparison with healthy ones. Also, the study comprises the effect of bacterial respiratory affection of camels on blood gases and acid-base balance. There were a marked decrease in blood pH value and base excess (B.E), while significant decrease in Po_2 value as well as there is significant increase in PCo_2 value in clinically diseased camels in comparison with healthy ones. Antibiotic sensitivity tests for the isolated bacteria revealed that Garamycin is the best sensitive antibiotic of choice.

Key works: *Bacterial causes, camels, respiratory affection, antimicrobial susceptibility, bood gases and acid-base balance, Assiut.*

INTRODUCTION

Camel is an animal of considerable importance in Egypt since it is one of the major sources of meat production while its meat represents 66.46% of the total meat obtained from the imported animals for slaughter purposes, Anon, (1986) and Gobrial, *et al.* (1991). Also it has a great value among our farm animals, working in agriculture fields or traveling between the villages and carrying out the farmers crops.

Respiratory diseases of camels continue to be a major cause of economic losses and adverse in animal. Stress of cold weather, rain, bad

hygiene and high humidity rate were incriminated to increase the respiratory infection (Bacterial, viral and parasitic). Respiratory affection is the main cause of death among camel calves allover the world (Chowdhary, 1986 and Khanna *et al.,* 1992).

Bekele (2004) recorded that the major clinical signs observed in camels suffering from respiratory disease were cough, loss of appetite and watery nasal discharge which became mucoid.

Bacterial infection of the lung is one of the main causes of pneumonia in camels (Rana *et al.,* 1993; Thabet, 1993; Alhendi, 2000 and Seddek, 2002).

Several species of micro organisms could be isolated from both apparently healthy and affected respiratory tract camel such as Staphylococci, Streptococci, *E.coli*, Pasteurella and Klebsiella (Ghawi, 1978; Rana *et al.,* 1993; Fatma *et al.,* 2001 and Seddek, 2002).

Arora and Kalara (1973) mentioned that Streptococci, Staphylococci, *E.coli* and Klebsiella spp. Were the predominant isolates from respiratory tract infection in camels.

Ismail *et al.* (2008) recorded that the most prevalent bacteria among respiratory affection of camels was, Staphlococcus aureus.

Azam and Zaki (2006) reported that the principle causes in pneumonia in camels were Staphylococcus aureus, *E. coli*, Streptococcus pyogenes and P. multocida.

Tarazi (2001) recorded that Streptococci, Staphylococcus aureus, *E. coli* and Klebsiella spp. were the most frequent isolates from cases of pneumonia in one humped camels in Jordan.

Vashishta and Singh (1977) stated that Streptococcal pneumonia due to presence of streptococci in lung abscesses of the affected camels.

Vitovec and Vladik (1983) recorded that the Streptococci organismed was isolated from bronchial diseases of camels in Somalia.

El-Magawry *et al.* (1986) found that the most bacterial agent causing respiratory affection in camels were Staph. aureus.

Respiratory affections affect either directly or indirectly on some serum biochemical parameters. Oxygenation of the blood and gaseous exchange between the blood and lung tissue is the main function of the lung i.e., regulation of the oxygen tension and carbon dioxide concentration.

In pneumonia and bronchitis, there were a marked increase in pressure Co_2 (PCo_2) values associated with drop in blood pH values (El-Sebaie *et al.,* 1988). The effect of acidosis are related chiefly to the respiratory system. Increased carbon dioxide tension of the blood and

depletions of bicarbonates caused an increase in the depth and the rate of respiration by stimulation of the respiratory centre (Radostits *et al.,* 2002).

MATERIALS and METHODS

Animals:

A total of 90 camels were used in this study which slaughtered at Assiut governorate abattoir in Bani-Adi. The age of slaughtered camels ranged from 2-5 years, 25 camels showing signs of respiratory disturbances including moist cough, rapid breathing and watery to mucoid nasal discharge, and the rest of camels were apparently healthy (65) used as control.

Bacteriological examination:

65 Nasal swabs from both apparently healthy and diseased camels were collected before slaughtering. The collected swabs were inoculated into nutrient broth and incubated at 37°C for 24 hr. After slaughtering 75 samples from the lung, pulmonary lymph nodes and pleural fluid were taken from clinically diseased camels under a septic conditions and sent without delay to the laboratory, then inoculated on nutrient broth for 24h at 37°C. Loopful was taken from the inoculated broth and recultured on nutrient agar, 5% sheep blood agar, Manitol Salt agar and MacConkeys agar then incubated at 37°C for 24-48 hr.

The isolates were identified according to colonial morphology, pigment production, microscopically by Gram stain and biochemically according to Bailey and Scott (1974) using catalase activity, coagulase as well as novobiocin (30 mcg) and polymixin sulphate (300 μ) senstivity test for identification of Staphylococcus sp.

Antimicrobial susceptibility tests of isolates:

Antibiotic sensitivity tests were done for bacterial isolates using antibiotic disks (Biomerieux) of Ampicillin (10 μg), chloramphenicol (30 μg), Erythromycin (15 μg), Garamycin (30 μg), Oxytetracycline (30 μg), Kanamycin (30 μg), Neomycin (30 μg), Spectinomycin (20 μg), Tetracycline (30 μg), and Nalidixicc acid (30 μg) according the method of Koneman *et al.* (1997), while, zones of inhibition were determined according to National Committee For Clinical Laboratory Standards (NCCLS, 2002).

Blood samples:

10 blood samples were taken from both healthy and diseased camels for serum biochemical analysis of copper (µg/dL) and Iron (µg/dL) levels were estimated colorimetrically using Digital ultraviolet spectrophotometer Model 292 by means of test kits supplied by Boehringer Manuhiem Gmbh Diagnostica after the methods described by Trinder, (1956) and Zac (1958) respectively.

Another blood samples with anticoagulant 1: 1000 sodium heparin were collected from both clinically healthy and diseased camels for determination of blood pH, PCo_2, Po_2, HCo_3, TCo_2 and B.E by using corning pH – blood gas analyzer Model 168. The analyzer directly measured at 37°C.

Statistical analysis of obtained serum biochemical data was preformed according to method of Snedecor and Cochran (1980) using test.

RESULTS

All results were illustrated in Tables (1-5).

Table 1: Incidence of positive bacterial isolates from respiratory tract of slaughtered camel samples.

Types of samples	Conditions								
	Apparently healthy			Diseased			Total		
	No.	Positive		No.	Positive		No.	Positive	
		No.	%		No.	%		No.	%
Nasal swabs	65	20	30.76	25	25	100	90	45	50
Lung	-	-	-	25	24	96	25	24	96
Pulmonary lymph nodes	-	-	-	25	20	80	25	20	80
Pleural fluid	-	-	-	25	14	56	25	14	56
Total	65	20	30.76	100	83	83	165	103	62.42

Table 2: The isolated micro-organisms of both apparently normal and respiratory diseased camels.

Isolated micro-organisms	Total number of isolates	% from total number of isolates	Nasal swabs				Diseased camels					
			Apparently healthy		Diseased camels		Lung		Pulmonary lymph nodes		Pleural fluid	
			No.	%	No.	%	No.	%	No.	%	No.	%
Staph. aureus	47	45.63	--	--	14	13.59	12	11.65	12	11.65	9	8.74
Staph. epidermidis	17	16.50	16	15.53	1	0.97	--	--	--	--	--	--
Strept. Pyogenes	17	16.50	--	--	6	5.82	4	3.88	5	4.85	2	1.94
E.coli	20	19.42	4 (untypable)	3.38	4 (O_{126}/B_6)	3.88	5 (O_{126}/B_6) / 1 untypable	4.85 0.97	3 (O_{126}/B_6)	2.91	3 (O_{126}/B_6)	2.91
Klebsiella pneumonia	2	1.94	--	--	--	--	2	1.94	--		--	--
	103	99.99	20	19.42	25	24.27	24	23.30	20	19.41	14	13.59

Table 3: Inhibition Zone diameter (IZD) of test antibiotics against isolates of micro organisms

Antibiotics	Isolated micro-organisms				
	Staph. aureus	Staph. epidermidis	Strept. Pyogenes	Klebsiella sp.	E.coli sp.
Tetracycline (30 µg)	R(<14 mm)	I(<17 mm)	R(<14 mm)	R(<14 mm)	R(<14 mm)
Erythromycin (13 µg)	S(>18 mm)	S(>18 mm)	S(>18 mm)	R(<13 mm)	R(<13 mm)
Garamycin (30 µg)	S(>15 mm)	S(>15 mm)	S(>15 mm)	S(>15 mm)	S(>15 mm)
Neomycin (30 µg)	R(<10 mm)	R(<10 mm)	R(<10 mm)	R(<10 mm)	R(<10 mm)
Kanamycin (30 µg)	R(<10 mm)	R(<10 mm)	R(<10 mm)	I(<17 mm)	I(<17 mm)
Chloramphenicol (30 µg)	R(<12mm)	R(<12 mm)	R(<12 mm)	R(<12 mm)	I(<17 mm)
Oxytotracycline (30 µg)	I(<17 mm)	S(>14 mm)	I(<17 mm)	R(<13 mm)	R(<13 mm)
Ampicillin (10 µg)	I(<25 mm)	I(<25 mm)	I(<25 mm)	S(>29 mm)	I(<13 mm)
Spectinomycin (20 µg)	R(<11 mm)	R(<11 mm)	R(<11 mm)	R(<11 mm)	I(<14 mm)
Nalidixic acid (30 µg)	R(<13 mm)	R(<13 mm)	I(<17 mm)	R(<13 mm)	R(<13 mm)

R: Resistant
I: Intermediate
S: Sensitive

Table 4: Mean values ± standerd deviation of blood serum iron and copper levels diseased camels.

Animals	No.	Fe.(µg/dL)	Cu.(µg/dL)
Apparently healthy	10	120.40±2.13	129.60±3.33
Respiratory infected	15	76.50±12.37*	257.76±40.22**

* Significant ($p<0.05$) ** highly significant ($p<0.01$)

Table 5: Overall values of blood gases and acid-base balance in clinically healthy and diseased camels.

Groups of animals	N	pH	Pco_2 mm Hg	Po_2 mm Hg	HCo_3 mmol/L	TCo_2 mmol/L	B.E. mmol/L
Apparently healthy	10	7.362±0.007	59.28±2.79	27.27±1.33	35.6±1.75	36.81±1.83	8.176±1.43
Camels with respiratory affection	15	7.231±0.067**	62.43±3.80*	22.01±1.43*	32.5±1.56	34.6±1.62	2.58±1.31**

N = Number of camels.
± S.D. = Standard deviation
* = Significant ($P < 0.05$)
** = Highly significant ($P < 0.01$)

DISCUSSION

The most clinical signs of diseased camels with respiratory affection were moist cough, anorexia, depression, loss of apetite, harried respiration and watery to mucoid nasal discharge. These findings agree with Abd-El-Kader, (1992) and Bekele, (2004).

In tables 2 the nasal bacterial swabs isolated from apparently healthy camels were Staphylococcus epidrmides 16 (15.53%) and *E.coli* 4 (3.88%). These results indicated that the respiratory tract of apparently healthy camels acted as a reservoir for many species of pathogenic and potential pathogenic microorganisms, Stress factors such as changes in the hygienic environmental and climatic conditions play a role in the onset of prenmonia (Phillip, 1972; Buxton and Fraser, 1977) Abd El-Kader (1992), was supported in the present study by the fact of the number of bacteria were isolated from apparently healthy camels and agreed with our data because the author was isolated Staph. epidermides and *E.coli* from nasal swabs of clinically healthy camels. Phillip, (1972) recorded that the bacterial isolated from the nose of apparently healthy camels were Staph. epidermides and *E.coli*. these data indicated that the animals were harboring these micro-organisms as members of nasal microflora.

In Table 2 the obtained results revealed that the total number of bacterial isolates from nose of diseased camel 25 (24.27%). The isolates were 14 (13.59%) Staph. aureus, 1 (0.97%) Staph. epidermides, 6 (5.82%) Strept. pyogenes and 4 (3.88%) *E.coli* (O_{126}/B_6). A variety of pathogenic and potentially pathogenic bacterial isolates from examined samples with variable incidence and frequency percentages.

Also, Ismail *et al.* (2008) agreed with our data. the authors concluded that the major prevalent bacteria among respiratory affected cases were Staph. aureus. These data disagree with Bekele, (2004), who said that the major clinical signs of pneumonia in camels was due to pasteurella haemolytica, Also, Azam and Zaki, (2006) recorded that the isolated strains from pneumonia in camels were Staph. aureus, Strept. pyogenes, *E.Coli*, P.multocida and P.haemolytica. The obtained results in table 2 revealed that most bacterial isolates from pulmonary lymph nodes and pleural fluid were 12 (11.65%) Staph. aureus; 5(4.85%) Strept. pyogenes; 3(2.91%) *E.coli* (O_{126}/B_6) and 9 (8.7%) Staph. aureus; 2 (1.94%) Strept. pyogenes; 3 (2.91%) *E.coli* (O_{126}/B_6) respectively. These data are nearly similar with Moustafa (2004) who isolated Staph. aureus 1 (5%); 2 (8.33%); *E.coli* 1 (5%); 1 (4.16%) and Strept. pyogenes 3 (15%); 1 (4.16%) from lung and pulmonary lymph nodes respectively from slaughtered pneumonic camels. From these previous data one could

observe that the bacterial isolates of the nose were more than that isolated from the lungs, pulmonary lympnodes and pleural fluid because of the direct contact of nose with environmental conditions and also the local antibodies, producted by lymphpcytes of the lung overcome such microbial agents.

Antibiotic sensitivity tests for the isolated bacteria revealed that the Garamycin and Ampicillin were the most effective antibiotic. For the isolated bacteria which representive in table 3 these findings were in agreement with Thabet, (1993) and Seddek, (2002). While Bekele, (2004) disagree with obtained data, he reported that Oxytetra-cycline was more effective in the treatment of respiratory diseased camels while Staphylococcus sp. and streptococcus pyogenes were highly sensitives to Erythromycin.

Cheyne, *et al.* (1977) reported that an apparent improvement of the pneumonic camels following intramuscular injection of Oxytetracline. It had been suggested that resistance of bacterial isolates to some antibiotics may be attributed to wrong dosage, duration of treatment and route of administration (Amstutz *et al.*, 1982). On the other hand Azam and Zaki (2006) mentioned that camel affected with bacterial respiratory affection (Staph.aureus, *E.coli*, Strept. pyogentes, Pastruella multocida) were highly sensitive to Gentamycin followed by Nitro furantion, Amikacin, Enrofloxacin and Choramphenicol, respectively.

Finally respiratory disorders is still serious problem due to its special property that multifactors are responsible and the difficulty to determine the definite cause, so more efforts must be done to overcome that problem, such efforts as periodical, clinical and bacteriological examination of apparently healthy animals to avoid misuse of antibiotics.

In Table 4 the value of blood serum iron (120.40±2.13 mg/dl) and copper (129.60±3.33 mg/dl) in clinically healthy camels were in agree with these recorded by Abd-El-Kader, (1992). El-Magawary *et al.* (1986) they mentioned that the mean value of blood serum iron in normal dromedary camel was 120.4±2.15. While El-Amrousi *et al.* (1984) mentioned that the main value of copper in blood serum of female healthy camel was 110.9±7.82. On the other hand, there was highly significant ($p<0.01$) decrease in iron level and highly significant increase in copper level in diseased camel in comparison with healthy ones. These results were similar to that obtained by El-Magawry *et al.* (1986) and Abdel-Kader, (1992). The reduction of blood trace elements mainly to impaired absorption or increased excetion of respective element. Kaneko and Crnelius, (1970) recorded the loss of appetite and loss of blood and the main general causes

of these blood trace elements deficiency which accompany bacterial and parasitic diseases.

Ghergariu and Kadar (1979) mentioned that there was a signicicant decrease in iron serum level of calves affected with preumonia, while an increase in serum copper level was recorded. On the other hand, Schulz *et al.* (1987) disagreed with our data, where the author recorded that there were no changes in iron and ketones in calves with pneumonia.

Abdel Kader, (1992) recorded that changes take place in levels of iron and copper associated with pneumonia in camels.

In preumonia and bronchitis, there were a marked increase in PCo_2 values associated with drop in blood pH values (Rosenberger, 1979 and Sodeman, 1961). The effect of acidosis are related chiefly to the respiratory system. The increased carbon dioxide tension of the blood and depletions of bicarbonate causes an increase in depth and the rate of respiration by stimulation of the respiratory center (Radostits *et al.*, 2002). Respiratory acidosis may occur in animals during pneumonia (Dukes, 1964)

The values of blood gases and acid-base balance in clinically healthy and diseased camels in Table 5.

The values of blood gases acid-base balance (pH, PCo_2, Po_2, HCo_3, TCo_2 and base excess) in clinically healthy camels were in agreement with values recorded by Habashy, (1985). In clinically diseased camels where there were highly significant decrease ($p<0.01$) in value of pH and base excess (B.E) (7.231 and 2.58 mmol/l) while there was a significant ($p<0.05$) decrease in value of Po_2 (22.01 mmHg) and significant ($p<0.05$) increase of PCo_2 (62.43 mmHg).

These obtained results nearly similar with previously results recorded by El-Sebaie *et al.* (1988) mentioned that pneumonia of calves associated with marked drop in blood pH and B.E while there were highly increase in values of PCo_2 and significant decrease in Po_2.

The drop of pH values and base excess in diseased camels due to respiratory acidosis and this finding were in agreement with data after Coles, (1980) and Haskins, (1983).

Also, a significant increase in power co_2 and a significant decrease in O_2 tension could be attributed to defect in oxygenation process of the lung during the course of pneumonia which leads to retention of Co_2 in blood (Coles, 1980). Respiratory acidosis (Coles, 1986) occur when Co_2 elimination is decreased, and blood carbonic acid concentration and PCo_2 are increased. Other causes of respiratory acidosis include intrathrocic and preumonia.

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