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BOVINE EPHEMERAL FEVER: ISOLATION OF THE CAUSATIVE VIRUS AND THE ASSOCIATING BACTERIAL RESPIRATORY COMPLICATIONS

(With 8 Tables)

By

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حمى الثلاثة أيام في الأبقار: عزل الفيروس المسبب والبكتريا المصاحبة للمضاعفات التنفسية

عبد الرحمن عبد المجيد عبد الرحمن ، عرفات صادق سيد ، على حسن صديق ، نوال محمد على

مع بداية صيف عام ٢٠٠٠ أصيبت قطعان الماشية في معظم محافظات جمهورية مصر العربية بمرض حمى الثلاثة أيام. أجريت هذه الدراسة على عدد ٥٨٥ حيوان من الماشية الفريزيان في محطتي بنى مر وأبنوب الحمام بمحافظة أسيوط. ظهرت الأعراض الإكلينيكية على عدد ٢٦٧ بقرة (٤٥,٦%) وقد وصلت نسبة النفوق و الذبح الاضطراري بين الحيوانات المريضة إلى ٩,٣% . استهدفت الدراسة عزل الفيروس المسبب للمرض وكذلك عزل وتصنيف البكتيريا المصاحبة للأعراض التنفسية. أظهر الفحص الإكلينيكي للحيوانات المريضة ارتفاع حاد ومفاجئ في درجة حرارة الجسم مع زيادة إفراز وارتجاع اللعاب ورشح أنفي تراوحت حدته بين الحيوانات المختلفة ما بين المصلى الشفاف إلي المخاطي أو المخاطي الصد يدي . ظهرت على الحيوانات العديد من الأعراض المصاحبة للمرض مثل وجود امفزيما تحت الجلد بدرجات متفاوتة تراوحت بين البسيطة إلي الشديدة في مناطق الرأس والرقبة والظهر وبين الضلوع، كما شوهد وجود أوديما اسفل الفك السفلي امتدت إلي اللباب والرقبة والصدر ووجود تورم وتصلب في العضلات والمفاصل وعتامة في قرنية العينين . لوحظ تضخم الغدد الليمفاوية و زيادة في معدلات الإصابة بالتهاب الضرع . تبين أيضا زيادة شدة الأعراض التنفسية المصاحبة للمرض و ارتفاع نسبة النفوق عن المعدلات المسجلة من قبل. تم عزل الفيروس المسبب للمرض في ٥٠ % من الحالات المصابة و ذلك عند حقن عينات من كريات الدم البيضاء للحيوانات المريضة في فئران التجارب وتم عزل الفيروس أيضا في نسبة ١٤,٣٢% عند زراعة عينات من كريات الدم البيضاء على الأنسجة الحية وتم التعرف على الفيروس عن طريق الاختبارات

السيرولوجية المختلفة . أظهر الفحص البكتيريولوجى للمسحات المأخوذة من الحيوانات السليمة و المريضة والنافقة وجود عدوى بكتيرية بنسبة٦١,٣٣ % ، كان من بينها عدد ٢٤٦ عدوى بكتيرية منفردة بنسبة ٤٨,٠٥ % و ٦٨ عدوى بكتيرية مختلطة بنسبة ١٣,٢٨ % . تم عزل الميكروبات الآتية: باستيريلا مالتوسيدا، باستيريلا هيموليتيكا، هيموفيلس سيمنس، استافيلوكوكس اوريوس، استافيلوكوكس ابيدرميدز، استربتوكوكس بيوجينس، نيموكوكس، كوراين باكتريم بيوجينس، كليبسيللا و الاشيريشيا كولاى وسيتروباكتر وسيدوموناس بنسب مختلفة . من خلال الدراسة المعملية للعترات المعزولة من حيوانات التجارب وجد أن عترات الباستيريلا والهيموفيلس كانت شديدة الضراوة على الفئران البيضاء . تم عمل اختبار الحساسية للعترات المعزولة باستخدام المضادات الحيوية المختلفة واتضح من خلال النتائج السابقة أن السبب الرئيسي للمضاعفات التنفسية المصاحبة لحمى الثلاثة أيام هو ميكروبات الباستيريلا مالتوسيدا وباستيريلا هيموليتيكا و هيموفيلس سيمنس وكورين بكتريم بيوجينس ونيموكوس .

SUMMERY

With the beginning of summer 2000, an outbreak of Bovine ephemeral fever (BEF) had affected cattle and buffaloes allover Egypt. This study was carried out on 585 animals in Beni-Morr & Abnoub Holstein Friesian dairy stations -Assiut Governorate. Clinical signs were evident in 267 Cows (45.6%) and the rate of deaths & emergency slaughtered animals reached 9.36 %. The main clinical signs observed were sudden and severe fever, increased salivary secretions and serous to mucopurrulent nasal discharges. The respiratory signs were severe and progressive. Clinical examination revealed many associating signs such as mild to huge subcutaneous emphysema in the regions of head, neck, back and intercostal muscles. Swelling and rigidity of the skeletal muscles and joints, corneal opacity, enlarged lymph nodes and increasing incidence of acute mastitis were observed. Isolation of the causative virus and associating bacterial complications were carried out. The causative virus was isolated and identified in 50 & 32.14 % of the examined samples by both mouse inoculation and tissue culture respectively. Bacteriological examination of swabs collected from nasopharynx, trachea and lung tissues revealed that 314 samples (61.33%) were positive. Single bacterial infection was detected in 48.05%, however mixed infection was recognized in 13.28% of the positive cases. The isolated bacteria were *Staph. aureus, Staph. epidermidis, Strept. pyogenes, Pneumonococcus* spp. *C. pyogenes, Pasteurella heamolytica, Pasteurella multocida, Klebsiella pneumoniae, Haemophilus somnous, E.coli, Citrobacter* spp. and *Pseudomonas*

aeurogenosa. All isolates of *Pasteurella* spp. and *Haemophilus somnous* were highly virulent to mice within 3-6 days after intraperitoneal injection with 7.5 $x10^6$ viable organisms. Antibiotic sensitivity test for the obtained isolates was carried out. The study concluded that, the respiratory complications such as pneumonia and fatal plumonary emphysema associating BEF could be attributed to secondary bacterial infection especially *Pasteurella* spp., *Hemophilus somnus, C. pyogens* and *Pneumococcus* spp.

Key word: *Bovine, Ephemeral, Fever, Respiratory complications.*

INTRODUCTION

An outbreak of Bovine ephemeral fever (BEF) among cattle had been recorded in summer 1991 in the different Governorates of Egypt. The virus antigen was detected in the leukocytes of the infected animals by using the indirect immunoflurescence technique (Hassan *et al.*, 1991). During the same outbreak, the virus has been isolated by intracereberal inoculation of baby mice and in baby hamester kidney (BHK21) cell culture (Soheir, 1994). During summer 2000, clinical and epidemiological investigations of Bovine ephemeral fever has been recorded in Egypt (Zaghawa et. al., 2000 and Sayed *et al.*, 2001). BEF is an arthropod-born viral disease of cattle and water buffaloes characterized by acute fever, stiffness, lameness and nasal discharges. The disease may be followed by various complications such as pneumonia, subcutaneous and pulmonary emphysema (Theodoridis and Coetzer, 1979; St George, 1988; Nagano *et al.*, 1990 and Farag *et al.*, 1998).

One postulated mechanism of viral-bacterial synergism is that of epithelial damage of respiratory tract by viruses allow the penetration of bacteria, which would normally be cleared by host defense (Loosli, 1968). The interactions of pulmonary, viral, and bacterial infections have been studied experimentally in mice in which, bacterial pneumonia is enhanced by prior viral induced impairment of clearance (Degre and Solberg (1971). Viruses and mycoplasma primarily play role in upsetting the defense mechanism of the animals, while bacteria and their toxins play a crucial role in the development of pulmonary lesion (Trigo *et al.*, 1984)

Stress resulting from transportation and latent viral infection allows pathogenic bacteria, primarily *pasteurella species* to invade the

lower respiratory tract and release of bacterial toxin, resulting in congestion and edema and over time this leads to fibrinous pleuritis with necrosis and abcessation of cranioventral lobes (Wilson *et al.*, 1985 and Andrews *et al.*, 1992). Markhan and Wilkie (1980) suggested that the role of virus and other factors might be that of impairing alveolar macrophage function sufficiently to allow *pasteurella hemolytica* to proliferate, since greater number of the bacteria cause further macrophage dysfunction and eventual cytotoxicity

The most common causes of respiratory troubles were refereed to *pasteurella* spp, *Corynebacterium* spp, *Streptococci* spp, *Pseudomonas* spp, *E. coli and mycoplasma bovis* (Collier, 1969; Elyas, 1982 and Vestweber *et al.*, 1990). *Pasteurella multocida, Pasteurella hemolytic, Streptococcus* spp., *Staphylococcus* spp., *Pneumoncocci, Pseudomonas* spp; *Clostridium perferengence, Corynebacteria* spp; *Klebisiella* spp; *Hemophilus* spp *and E.coli* were isolated from nasal mucosa and trachea of healthy cattle (Handy and Tropp, 1967 & Singh and Malik, 1968) and from pneumonic cattle and buffaloes (Haritani *et al.*, 1990 and El- Sayed *et al.*, 1992).

This study aimed to isolate and identify the causative virus of Bovine ephemeral fever and the associating bacteria that may be responsible for respiratory complications.

MATERIALS and METHODS

I- Materials:

1-Animals:

A total number of 585 Friesian cattle of both sexes, belonging to Bani-Morr and Abnoub Holstein Friesian stations- Assiut Governorate were used in this study. The age of these animals varied from 2 months - 12 years. Signs of BEF and respiratory complications were evident in 267 animals, 25 animals were found dead and 293 animals were apparently healthy (Table 1). Clinical examination of these animals was carried out according to Radostits *et al.* (1994).

2-Samples: Two blood samples were collected from each diseased case as follow:

a- Whole blood samples with anticoagulant (EDTA) for virus isolation and identification.

b- Blood samples without anticoagulant for obtaining serum to estimate the antibody titer against BEF virus.

3- Antisera:

a- Reference antisera against BEF was kindly supplied by Plum Island Institute -USA.

b- Fluorescent antibovine immunoglobuline was prepared in rabbit - Difco, USA.

4-Virus: Reference BEF virus was kindly supplied by virology department, Fac. Vet. Med. Cairo University.

5- Laboratory animals: Suckling mice, 1-3 days old were used for virus isolation.

6-Tissue culture: Vero cells were used for virus isolation, serum neutralization and virus neutralization.

II- Adopted Methods*:

1-Blood samples preparation: Buffy coats were separated from the blood samples, which were collected on EDTA according to Davis and Walker (1974).

2- Virus isolation and identification, was carried out in the animal health research institute - El-Dokki, Dept of virology (Prof. Dr. N. A. MOHAMED)

a- Baby mice inoculation:

0.25 ml of diluted white blood cell suspension (1:10, v/v in minimal essential medium contains penicillin and streptomycin) was intracereberally (I/C) inoculated into suckling mice 1-3 days old. The mice were observed daily for any nervous manifestations or death. Impression smears were made from their brains for indirect fluorescent antibody technique according to Gardner and Quillin (1980).

b-Tissue culture: White blood cell of the previously prepared suspension was inoculated into confluent sheet of vero cell line and observed daily for the evidence of any cytopathic effect.

c-Serological examination:-Serum neutralization and virus neutralization tests using vero cells were carried out according to Carbery and Lee (1966).

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*: *The procedures of preparation of blood sample and virus isolation were carried out in Animal Research Institute - El-Dokki - Giza (Dr. Nawal M. Ali)*

3- Bacteriological examination:

A- Culturing:

Sterile swabs were collected from 487 animals of the examined cases (220 apparently healthy and 267 of diseased cattle). Another 25 sterile swabs were collected from affected trachea and lung of dead or slaughtered cattle. Sterile swabs were inoculated into sterile brain heart infusion broth and incubated at 37^0C for 24 hours. Loopful of brain heart infusion broth were recultured on the respective media according to Cruickshank *et al.* (1975), Carter (1984) and Baily and Scott (1994) as follow:

a- Nutrient agar plates were used for isolation of different microorganisms and demonstration of produced pigments.

b- 5% sheep blood agar plates and 10% chocolate blood agar plates were used for isolation and differentiation of the hemolytic and non hemolytic microorganisms, *pasteurella* spp. and delicate microorganisms especially *pneumococci.*

c- Taurillat blood agar was used for isolation of *Corynebacterium* spp.

d- Brain heart infusion agar with 10% bovine blood and 5% yeast extracts under 5-10% Co_2 for 3 days at 37^0C was used for isolation of *Hemophilus somnus* organisms.

e- MacConkys agar plates were used for isolation of enterobacteriaece. Cultivated plates were examined after 24 - 48 hours incubation at 37^0C for bacterial growth.

B- Microscopical examination:

Blood films were made from peripheral blood vessel on clean dry sterile glass slide and stained with Giemsa stain for direct microscopical examination to detect the presence of bipolar microorganisms. Also smears were prepared from different bacterial colonies and stained with Gram stains.

C- Biochemical reaction: -

Final identification of bacterial isolates was carried out by using the following tests: Motility test, catalase test, urease activity, gelatin liquefaction, indol test, coagulase test, fermentation of sugars, H_2S test, potassium cyanide test, methyl red test and Vogues Prauskauer test according to Cruickshank *et al.* (1975).

D- Laboratory animal inoculation: -

A total number of 125 white mice weighing 25-30 grams were used to investigate the pathogenicity of isolated *Pasteurella* spp. and

Hemophilus somnus, five mice were used for each isolate according to Carter (1984). Isolated *Pasteurella* spp. *and Haemophilus somnus* were cultured on brain heart infusion broth at 37^0C for 24 hours. 0.5 ml ($7.5x10^6$ viable organism) of each cultured broth was inoculated intraperitoneal into mice, while the control mice were inoculated with sterile broth. The mice that died within 24 – 48 hours, were dissected, then blood films were made from the heart blood and stained with Giemsa stain for microscopical examination of the bipolarity of *Pasteurella* spp. Swabs from the liver tissues were taken and inoculated to insure further purification of the *pasteurella* microorganism.

E- Sensitivity test:

In vitro sensitivity test was performed for different isolated strains by the agar diffusion technique (Finegold and Martin, 1982). The antimicrobial activities of these isolates were tested on Muller Hinton agar containing 5% sheep blood. The plates were used within 7 days of preparation. One ml of trypticase soya broth (BBL) inoculated with 3-5 isolated colonies was incubated at 37^0C for two hours, 0.1 ml of this culture was spread over the surface of a plate and left to dry for 15-30 minutes at 37^0C. Multidiscs consisting of 10 ug ampicillin, 30 ug chloramphenical, 2ug lincomycin, 30 ug oxytetracycline, 5 ug penicillin, 10 ug streptomycin, 25 ug of sulphmethoxazole trimethoprim, gentamycin, erythromycin and enrofloxcin were left at room temperature for 30 minutes and then placed carefully in the center of the inoculated plates which were then incubated at 37^0C for 24-48 hours. The zone of inhibition were measured and interpreted according to the method of Thoransberry and Baker (1981).

RESULTS

Results of morbidity, mortality, viral and bacterial isolation are illustrated in Tables 1-8.

Table 1: Morbidity and mortality rates of BEF among animals in Beni-Morr and Abnoub Holstein Friesian dairy stations.

No. of examined Cases	Apparent healthy		Diseased		Dead &slaughtered	
	No.	%	No.	morbidity (%)	No.	mortality (%)
585	293	50.09	267	45.6	25	9.36

Table 2: BEF virus isolation from the buffy coats of viramic cattle in baby mice brain and Vero cell live

Locality	No. of samples	Baby mice brain		Vero cell	
		+Ve	%	+Ve	%
Bani-Morr	16	9	56.25%	6	37.5%
Abnoub	12	5	41.66%	3	25%
Total	28	14	50%	9	32.14%

Table 3: Neutralization antibody titer against BEF in the sera of infected Cattle

Locality	No. of tested sera	No. of +Ve sera	% of +ve cases	Neutrilizing antibody titre				
Bani-Morr	14	11	45.8	4	8	16	32	64
Abnoub	10	7	29.2	3	0	4	3	1
Total	24	18	75	2	0	3	2	0

Table 4: The number and perventage of positive bacterial cases in the examined group of animals.

Condition of animal	Types of samples					
	Nasopharyngeal swabs			Trachea and lung tissues		
	No.	+ve	%	No.	+ve	%
Apparently healthy	220	60	27.27	-	-	-
Diseased	267	231	86.52	-	-	-
Dead and slaughtered	-	-	-	25	23	92.00
Total	487	291	59.75	25	23	92.00

Table 5: Incidence of single and mixed bacterial infection in the examined groups of animals

Type of infection	App. healthy (220)		Diseased (267)		Dead & slaughtered (25)		Total (512)	
	No.	%	No.	%	No.	%	No.	%
Single infection	45	20.45	195	73.03	6	24	246	48.05
Mixed infection	15	6.82	36	13.48	17	68	68	13.28
Total	60	27.27	231	86.51	23	92	314	61.33

Table 6: Microorganisms isolated from positive cases with mixed infection in different groups of cattle.

Types of swabs / Mixed isolates	Nasopharyngeal		Trachea & lung	Total	
	Apparent health	Diseased	Dead & Slaughtered	No.	%
Staph.aureus+Past.haemolytica	2	9	5	16	23.53
Strept. Pyogn+Corynebacterium	3	4	3	10	14.71
Pneumoncocci+Past.haemolytica	2	5	4	11	16.18
Past.multocida+Strept.pyogenes	2	4	2	8	11.76
Haemophilus somnous+ Staph.aureus	1	6	1	8	11.76
Klebsiella pneumoniae+E.coli	4	5	0	9	13.24
Strpt. Pneumonia+Haemophilus somnous	1	3	2	6	8.82
Total	15	36	17	68	100

Table 7: Microorganisms isolated from positive cases with single infection in different groups of cattle.

Types of swabs / Single isolates	Nasopharyngeal		Tracheal and lung tissues	Total	
	Apparantly healthy	Diseased	Dead & Slaughtered	No.	%
Staph. aureus	4	10	0	14	5.7
Staph. epidermidis	9	4	0	13	5.3
Strept. Pyogenes	3	18	0	21	8.5
Pneumococcus	5	19	1	25	10.2
Coryn. Pyogens	4	29	1	34	13.8
Past. Haemolytica	1	35	2	38	15.4
Past. Multocida	2	26	1	29	11.8
Kl. Pneumoniae	5	19	0	24	9.8
H.. somnus	0	22	1	23	9.4
E.coli	7	5	0	12	4.9
Citrobacter sp.	4	3	0	7	2.8
Pseud. Aeuroginosa	1	5	0	6	2.4
Total	45	195	6	246	100

Table 8: Antibiotic sensitivity tests for the bacterial isolates recovered from infected animals

Antibiotic	Type of isolates	
	Grams +ve	Grams -ve
Chloramphenicol	-	+++
Oxytetracyclin	+	++
Gentamycin	-	++
Erythromycin	++	+
Enrofloxcin	+++	++
Ampicillin	++	-
Penicillin	++	-
Trimethoprime sulphamethoxazol	++	+
Streptomycin	+	-
Lincomycin	-	+++

- Resistant
+Mild sensitivity
++ Moderate sensitivity
+++ highly sensitivity

DISCUSSION

Outbreak of BEF occurred in Egypt during the summer 2000. The infected dairy cattle showed clinical symptoms which characterized by sudden onset of fever, nasal and ocular discharges, hurried respiration, stiffness, lameness and subcutaneous emphysema with accumulation of air in the regions of head, neck and intercostal spaces in severe cases. Most of cows and bulls were infected with virus and showed signs of the disease, however no cases of young calves were affected. P.M. examination revealed signs of pulmonary emphysema, lung congestion, and petchial hemorrhages on pericardia and pleura. The same clinical symptoms were also described by Farag *et al.* (1998), Liao *et al.* (1998), Zaghawa *et al.* (2000) and Sayed *et al.*, (2001).

The morbidity and mortality rates were 45.6 and 9.36 % respectively (Table 1). BEF characterized by high morbidity and low mortality especially when little or no complication occurred. The obtained data lies within the range obtained by Martinez *et al.* (1987).

Isolation and identification of the causative agents:

a- Isolation of BEF virus:

The BEF virus was isolated and detected from buffy coat samples, where the virus affects the endothelium of small blood vesels

(Mackerras *et al.*, 1940). The virus is contained in the leukocyte fraction of the blood during fever (Theodoridis, 1969), and more particularly in neutrophils (Young and Spradbrow, 1980 and 1985). The BEF virus was isolated from the buffy coats of the collected 28 blood samples of the infected cattle. The isoaltion of the virus was carried out through inoculation of buffy coat into baby mice as well as into vero cells.

In Table 2, the results of attempts to isolate BEF virus from the buffy coat, prepared from blood of febrile cattle and intracereberally inoculated into suckling mice, 1-3 days-old revealed that the virus was detected in 14 isolates (50%). These results coincided with those reported by Van Der Westhuizen (1967), Inaba *et al.* (1968) and Doherty *et al.* (1969). On the other hand, the inoculated buffy coat in Vero cells revealed BEF virus in 9 cases (32.14%). This result more or less agreed with that recorded by Snowdon (1970). The number of virus isolates in tissue culture were less than that obtained by inoculation of suckling mice, that may be attributed to the inability of all strains of BEF virus to be readily adapted to Vero cells (Standfast *et al.* 1976).

b- Identification of the virus isolates:

The indirect fluorescent antibody test (IFAT) was applied on smears made from the brains of infected mice. Positive results show the presence of the granular fluorescence in the cytoplasm of the brain cells. Virus neutralization test was also applied to identify the isolates in Vero cell live.

c- The serological results:

Table 3 showed that, the antibody titer of 24 tested serum samples of infected cattle was ranged from 4 to 64 iu/ml in 18 positive samples (75 %) . The very low or absence of titer in the early stage of the disease followed by rising titer in 5 - 14 days from the beginning of the disease were expected, that agreed with Burgess (1974). This study proved the occurrence of BEF virus in the examined disease cases in Egypt.

2- Bacteriological examination:

Finding of bacteriological investigations of 487 nasopharyngeal swabs revealed that 291 samples (59.75 %) were positive for secondary bacterial complication, in addition to 23 of 25 tracheal and lung tissue swabs (92 %) were found also positive for bacterial infection. Bacterial isolates were found to be high in diseased, dead and/or slaughtered cases in comparison with those isolated from apparently healthy one

(Table 4) confirming the suggestion of secondary bacterial infection in association with BEF Martineze *et al.* (1987), Healy *et al.* (1993) and Selim *et al.* (1998).

Investigated bacterial isolates were detected as single infection in 246 cases (48.05 %) and as mixed infection in 68 cases (13.28 %). Similar results had been reported by Ishino *et al.* (1979) and Martinez *et al.* (1987), however these results were higher than those obtained by Selim *et al.* (1998).

Mixed bacterial isolates were found as: *Past. haemolytica* in association with *Staph. aureus,* (23.53 %); *Pneumonococci* with *Past. Haemolytica* (16.18%) and *Hemophilus somnus* with *Staph.aureus* (11.76%). These findings agreed with that obtained by Selim *et al.* (1998).

Data presented in Table 7, proved that *Pasterulla hemolytica, Corynbacteria pyogens, Pasterulla multocida, Pneumococcus, Klebsiella pneumoniae, hemophilus somnus and Streptococcus pyogenes* were the most important pathogenic isolates with a total percentage of 15.45, 13.82, 11.79, 10.16, 9.75, 9.35 and 8.54 % respectively. Other isolates of minor health significance were also recovered with variable frequency percentages. Similar pathogens were isolated by Brylin (1986), El-Haenaeey *et al.* (1994), Walker *et al.* (1996) and Selim *et al.* (1998).

This study revealed the presence of *Past. hemolytica, Past. multocida, Hemophilus somnus, Corynobacterium pyogenes, Pneumococcus* and *Kl. Pneumoniae in* association with Bovine ephemeral fever in this outbreak. It is clear that the bacterial respiratory complications associatong Bovine ephmeral fever, resulted in high percentage of morbidity and mortality rates among the exposed animals, that agree with the postulated mechanism of viral-bacterial synergism, stating that epithelial damage of respiratory tract caused by virus, predispose bacterial peneteration, which would normally be cleared by host defense (Loosli, 1968 & Degre and Solberg, 1971). The fatal pulmonary emphysema could be attributed to secondary bacterial infection (St. George, 1988; Nagano *et al.*, 1990 and Farag *et al.*, 1998). Viral infection allows pathogenic bacteria, primarily *pasteurella species* to invade the lower respiratory tract and release of bacterial toxins, resulting in congestion and odema and consequently leads to fibrinous pleuritis with necrosis and abcessation of cranioventral lobes (Wilson *et al.*, 1985 and Andrews *et al.*, 1992). The pathogenicity of isolatd

Pasteurella spp. and *Hemophilus somnus* to white mice revealed that all isolates were highly pathogenic to mice after intraperitoneal injection with $7.5x10^6$ viable organisms, producing acute septicemia and death within 3-5 days. This agrees with the result obtained by Selim *et al.* (1998).

Antimicrobial susceptibility test was carried out for the obtained bacterial isolates (table 8). All gram-positive isolates were sensitive to Penicillin, Enrofloxcin, Ampicillin and Trimethoprime sulphamethazole. On other hand the gram-negative isolates were sensitive to Chloramphenicol, Lincomycin, Enrofloxcin, Gentamycin, Oxytetracycline, and Streptomycin. Nearly similar results were reported by El-Haenaeey *et al.* (1994) and Selim *et al.* (1998).

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