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## KLEBSIELLA PNEUMONIAE MASTITIS IN DAIRY COWS

(With 3 Tables and 3 Figures)

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إلتهاب الضرع بالكليبيلا في الأبقار الحلوب

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إستهدف هذا البحث توضيح الصورة الإكلينيكية لبعض الأبقار المصابة بالتهاب الضرع القمامري وعرفه السببات لهذا المرض في إحدى المزارع الخاصة بمحافظة أسيوط التي تستخدم بعض المخلفات مثل نشارة الخشب مع قش الأرز كحفرشة للأبقار وكذلك معرفة المضادات الحيوية المؤثرة على هذه السببات مع محاولة علاج بعض هذه الحالات هذا وقد إشمئ البحث على ٢٩ بقرة خليط بين سلالاتي الفريزيان والبلندي منها ٩ بقرات مصابة بالتهاب ضرع حاد . وقد أثبتت النتائج أن البقر المصاب يعاني من إلتهاب ضرع حاد وكان الربع المصاب يعساني من ورم شديد مصحوب بالم حاد وأن البعس المفرز عبارة عن مائل مائسي عديم الرائحة ومن أم الأعراض التي ظهرت على الحيوانات المصابة هبوط عام مع فقدان في الشهية ، كما أظهر الفحص الميكروبيولوجي لعينات اللبن وجود ميكروب الكليبيلا بنسبة عالية مع بعض الميكروبات السالبة لصيغة الجرام وقد تقلص دور الميكروبات الموجبة لصيغة الجرام ولم يتم عزل أي من الفطريات المرصدة ولا الميكوبلازما ، وأظهرت نتيجة إختبار الحساسية للميكروبات المعزولة أن دواء السيفوبيرازون ذو تأثير فعال ولكن عند محاولة العلاج بهذا الدواء وجد ثلاث حالات لم يستجيبوا له وإستجابت له حالتين ولذلك ينصح بعدم إستخدام المخلفات الخشبية مثل نشارة الخشب كحفرشة للأبقار لأنها ربما توفر بيئة صالحة لنمو بعض الميكروبات المسببة لإلتهاب الضرع ، ويجب غسل الضرع وتجفيفه بعناية للتخلص من الإخراجية المتعلقة به قبل الحليب ، وكذلك يجوز إستعمال الرمل بدلا من نشارة الخشب لتوفير التكاليف .

### SUMMARY

The aim of the present investigation was to clear up the clinical signs and possible causes of cows mastitis in small private farm use a saw dust as a cow's bedding with rice straw, antibiogram for isolated organisms and therapeutic trial for some infected cases. Of thirty nine examined dairy cows, nine were found to be suffering from signs of acute mastitis and the infected quarter was suffered from sensitive edematous swelling

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along streak canal rather than the base of quarter secreting odorless watery discolored milk. Supramammary lymph nodes, were not involved. General examinations revealed the loss of appetite and lethargy were prominent signs of diseased cows. Microbiological studies indicated that Klebsiella pneumoniae was found to be the predominant isolated bacteria whereas no mycotic pathogens nor mycoplasma were detected. Cefoperazone (30 ug) had strong inhibitory effect on all isolates (in vitro). Unfortunately, milk yield of three cases of cows subjected to trial's treatment by cefoperazone (locally and systematically) didn't return to its normal level although the udder regained its normal shape. On the other hand, two cases of infected cows were apparently completely returned to its normal conditions. The present investigation claimed the following: a) saw dust as a cow's bedding should be avoided because it's may act as a source of infection. b) Before milking, the udder should be washed and carefully dried to remove the contacted fecal matter. c) Sand can replace the saw dust as cow's bedding.

## INTRODUCTION

During the last few years, the economic importance of dairy farms is increased in many parts of the world particularly in developing countries, and at the same time udder's diseases of lactating animals are becoming more and more important. Bovine mastitis is one of the most important problems in our dairy farms especially in small private farms where hygienic measures and milking sanitation are often insufficient.

Many genera and species of pathogenic micro-organisms were incriminated as causal agents of severe mastitis of dairy cows. For accurate diagnosis and in order to reach a valuable specific treatment for mastitic cows, mastitic pathogens responsible for infection should be isolated and identified as expeditiously as possible. On the other hand, haphazard applications of antimastitic patient preparations drugs to infected cows without detection of mastitic pathogens may produce a vital chance for growth of some microorganisms as yeast (RHAMAN et al., 1983), fungal infection (MISRA and PANDA, 1986) or both infection (ZAITOUN et al., 1991). Moreover, unhygienic applications of antimastitic drugs may plays a partial role in spread of the mycoplasma mastitis in dairy cows (ZAITOUN, 1991).

Klebsiella pneumoniae either alone or in association with other organisms was encountered as a pathogenic agent responsible for severe mastitis in dairy cows (OZ et al., 1985 and CARTER, 1986). The fundamental purposes of this investigation was to clear up the following: (a) the clinical signs and possible causes of cows mastitis in a small private farm, (b) antibiogram for isolated strains, (c) therapeutic trial for some infected cases.

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## MATERIAL and METHODS

## A) Farm characteristics :

This investigation was carried out on a small private dairy farm consists of thirty nine mixed breed (friesian and native strains) cows located in Assiut Governorate. Cows were kept in a close barn and the major component of cow's bedding was composed of rice straw above wetness soil land. Sometime, due to financial factor, part of rice straw was replaced by saw dust. The cows were manually milked twice daily. Pre- and post- milking disinfections and other sanitary measures were not routinely applied and if it's do, before milking, teat dipping was applied without wasing the udder. Furthermore, dry cow therapy was not utilized.

## B) Samples collection :

Only dairy cows showing clear signs of clinical mastitis were subjected to this investigation. From each mastitic case, one milk smple was collected from each infected quarter(s) in sterile vial with aseptic precautions as methods described by AKL (1988). All collected samples were submitted to the laboratory for culturing procedure within two hours. Patient data, prior treatment, culling rate and other knowledges were enrolled. Seven milk samples were collected from seven apparently healthy cows (one composite milk sample was collected from each cow) and subjected to the same work.

## C- Culturing procedure :

Each collected milk sample was subjected to mycoplasmal, mycological and bacteriological examinations. For mycoplasmal examination, each samples was plated directly and indirectly on B medium of the formula described by ERNO and STIPKOVITS (1973) as the method described by ZAITOUN (1990). For mycological examination, a loopful of centrifuged sediment from each smaple was inoculated on Sabouraud's dextrose agar medium supplemented with penicillin at 20 units and streptomycin at 100 ug per ml. with and without cycloheximide and rice agar medium. The inoculated plates were incubated both at room temperature (25°C) and at 37°C with daily observation for four weeks (MISRA and PANDA, 1986). The isolated fungi were identified morphologically according to FRCY *et al.* (1974). On the other side, for bacteriological examination, loopful of centrifuged sediment from each milk sample were streaked onto 5% ovine blood agar, MacConkey's agar and Levine EMB ager plates and incubated aerobically at 37°C for 24-48 h. Bacterial colonies were identified morphologically and biochemically as the methods described by CRUICKSHANK *et al.* (1975) and CARTER (1986).

## D) Trial's treatment :

Therapeutic trial for some infected cases was done (Table 3) by using local and systemic application of choice drug cefobid (cefoperazone sodium - Pfizer S.A.L., Cairo, ARE under Authority of Pfizer Inc. USA) as results of antibiogram for isoalted strains (Table 2). Milk samples were recollected at seven days post treatment to determine the degree of effectiveness.

**RESULTS**

Of thirty nine investigated cows, nine cases were found to be suffering from signs of clinical mastitis. Bacteriological examination revealed that Klebsiella pneumoniae was predominant isolated bacteria from mastitic cases (Table 1 & Fig. 1) while no mycotic pathogens nor mycoplasma were detected. On the other hand, Corynebacterium bovis was isolated from five normal samples.

**A) Clinical signs :**

The infected cows showed signs of acute mastitis with reduction in milk secretion accompanied by anorexia and severe depression. Treatment of these cases was done previously by tetracycline (Mastalone udder infusion - Pfizer), one infusion per infected quarter once daily for three successive days. The herd's owner repeat the treatment again to such incurable cases by cloxacillin and neomycin (Cloxa-gel intramammary syringe - Virbac). However, this applications no response was noticed.

Clinical observations of infected cows were confined to one to three quarters. The infected quarter suffered from sensitive edematous swelling with hotness. This swelling was markedly observed along streak canal rather than the base of quarter and extended upward (Fig. 2a & 2b). Three infected quarters appeared somewhat inexorable. Supramammary lymph nodes were not involved.

Milk secretion appeared as odorless watery discolored milk. In some cases, it was appeared as slightly brownish in color with small particles of clotted milk.

General examinations revealed that inappetence and lethargy were prominent signs of diseased cows. The heart rate was somewhat weak & rapid and increased in number ( $70 \pm 5/m$ ) in comparison with apparently healthy cows in the same farm ( $54 \pm 3/m$ ), but regular. Temperature was slightly elevated ( $39.6 \pm 3^\circ C$ ) some cases. Two of infected cows were suffered from diarrhea and moderate signs of dehydration.

**B) Antibiogram :**

Results of antibiogram for isolated strains indicated that cefoperazone (30 ug) had inhibitory effect on isolated Klebsiella pneumoniae as well as other isolates while the other used drugs were ineffective with exception of gentamicin (10 ug) had slight effect (Table 2).

**C) Therapeutic Trial :**

In the third day of treatment period of infected cases, an improvement in the animals' general condition was observed (body temperature, appetite restored in four cases) and the pain of infected quarter subsided as did swelling. At the end of therapeutic course the udder had regained its normal appearance in four cases and Klebsiella pneumoniae was not re-isolated from collected milk samples after treatment although the milk yield didn't returned completely to its previous level in three cases.

## DISCUSSION

Klebsiella pneumoniae is one of coliform organisms, occurs widely in nature, responsible for many diseases in animals including severe mastitis (CARTER, 1986). Dung matter is considered a source of these organisms in the environment (OZ et al., 1985). Any control program of any type of mastitis, whatever, in dairy farm depends mainly upon two steps, the first one is prevention of teat contamination; and the second step is avoidance of the invasion phase, in other words avoidance the invasion of mastitic pathogens to the mammary gland through teat meatus. Both steps were misprision in our investigated farm where cows were kept in close barn with damp litter and bad drainage which depends mainly on hand collection

Teat dipping with efficient disinfection may be misused. Isolation of Corynebacterium bovis from most collected samples, either mastitic or apparently normal, supports our opinion where CARTER (1986) reported that Corynebacterium bovis is the organism frequently recovered from cow's milk. Routine disinfections has strong effect on the prevalence of this organism (BRAMLEY et al., 1976). Accordingly, isolation of this organism in high rate is considered as an indicator for efficiency of teat disinfections and hygienic sanitation.

According to history taking, part of rice straw of cow's bedding was replaced by wood's by-product. Wood's by-product was probably implicated as a source of Klebsiella infection (BRAMAN et al., 1973 and BRAMLEY & NEAVE, 1975). This may appears overwhelmingly the origin of infection.

Intramammary infections were reduced greatly by milking hygiene (WILSON and KINGWILL, 1975) particularly premilking sanitation. Poorly drainage, continuous contact between cows udder and contaminated damp litter as well as unhygienic hand milking facilitate the invasion phase of mastitic pathogens to the gland through teat meatus. this may give account for streak canal swelling rather than systemic invasion.

Most Gram negative organisms are sensitive to desiccation and commercial products (in vitro) commonly used for teat dipping (OZ et al., 985 and JONES, 1986). Therefore, we suggested that, Klebsiella pneumoniae is described as an "opportunistic pathogen" in cows mastitis where this organism is likely to be present in poorly drained environment.

Teat dipping play a vital role in control of streptococcus and staphylococcus mastitis because these organisms colonise teat duct before invading the udder. So, they are possibly transmitted from infected quarter to another at milking time through milker's hand. In contrast, coliform does not colonise the teat duct (OZ et al., 1985 and JONES, 1986).

In certain study, BRANDER (1973) indicated that Klebsiella pneumoniae after deposition on the teat orific of cow's udder, may be able to pass through the duct between milkings. This may revealed that mastitis due to Klebsiella pneumoniae infection or other coliform occurs between milkings where the effectiveness of teat dipping disinfection is much reduced.

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Milk yield of three infected cases failed to return to the normal level after trial's treatment. Most gram negative bacteria produce endotoxin distracting the invaded tissue (CARTER, 1986). This may help to explain the failing of responsiveness.

In conclusion, Klebsiella pneumoniae mastitis in dairy cows, when they do, it is difficult to cure by common antimastitic drugs used in veterinary practice may due to complete destruction of mammary cells. Bad hygienic measures and wood's by-products litter may act as a good predisposing factors for growth of that organism.

It is important to appreciate that (a) Sand as a bedding material for cow's farm is more suitable than saw dust or other wood's by-products in control of coliform organisms (BRAMLEY and NEAVE, 1975), (b) Before milking, udder should be completely washed by rinse water to remove the contact fecal matter and careful drying by clean towel to avoid the invasion phase of coliform organisms particularly in the stage directly after milking when the teat sphincter is relaxed. Udder washing without careful drying plays a role in coliform mastitis (JONES, 1986), (c) Teat dipping with suitable disinfection should be urgently applied after milking.

Coliform infections were more sensitive to teat dipping containing linear dodecyl benzene sulfonate than iodine solution (BENNETT, 1982). Furthermore, teat dipping without udder washing should be avoided since contact organic matter (feces) may interfere the inhibitory effect of disinfection.

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Tab. 1 : Isolated bacteria from mastitic milk of cows (9):

Nr. of cases	Bacterial isolates	% to all infected cases
3	Klebsiella pneumoniae Corynebacterium bovis	33.34
2	Klebsiella pneumoniae Staphylococcus aureus E. coli (inactive)	22.22
2	Kelbsiella pneumoniae Pseudomonas aeruginosa Corynebacterium bovis	22.22
1	E. coli (inactive) Staphylococcus aureus Corynebacterium bovis	11.11
1	Pseudomonas aeruginosa Enterobacter aerogenes $\alpha$ hemolytic streptococci	11.11

Frequency distribution of isolated strains (22) from mastitic milk of cows

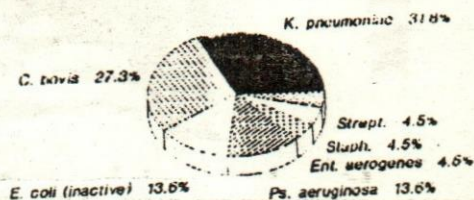


Fig. 1

Fig. 2a,2b: Marked swelling quarter of mastitic cows due to Klebsiella pneumoniae infection.

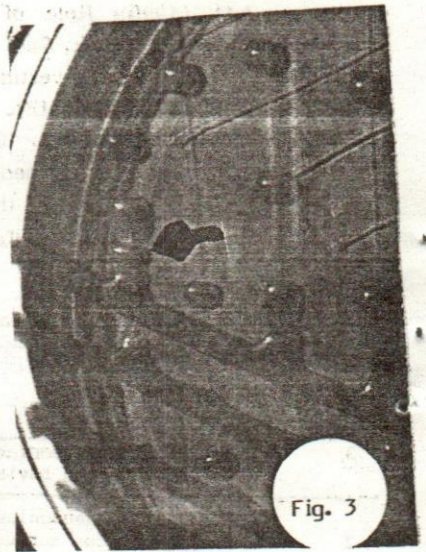
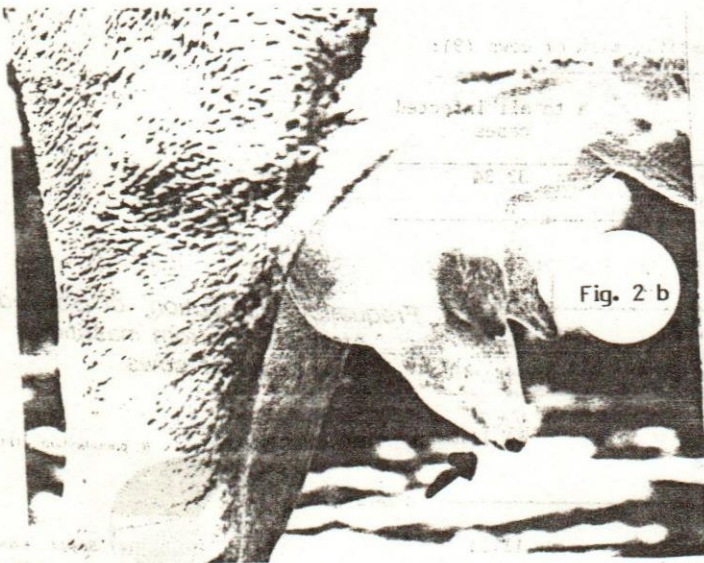
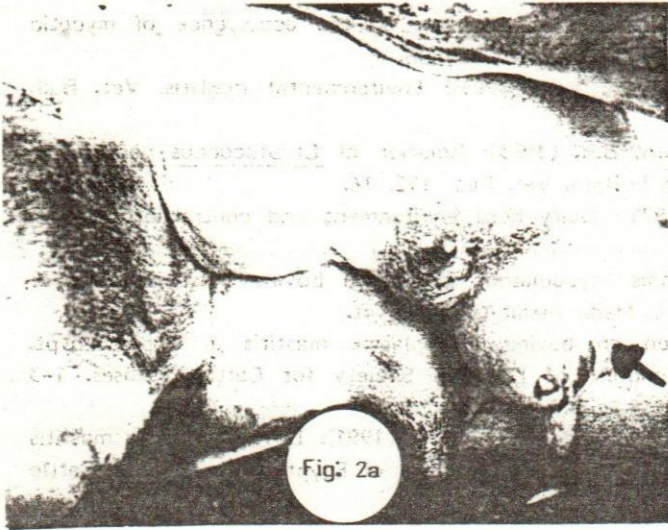


Fig. 3: Characteristic colonies of Klebsiella species on MacConkey's agar medium, Lactose fermenter and slime appearance.



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Tab. 2 : Antibiogram for isolated bacteria:

Drugs	Conc.	Source	<i>Klebsiella pneumoniae</i>	<i>Corynebacterium bovis</i>	<i>E. coli</i> (inactive)	<i>Pseudomonas aeruginosa</i>	<i>Enterobacter aerogenes</i>	<i>Staph. aureus</i>	Strept.
Ampicilline	25 µg	BioMerieux	-	+++	++	-	+	+++	+++
Amoxycillin	25 µg	BioMerieux	-	+++	+	-	-	+++	+++
Cefoperazone*	30 µg	Pfizer	+++	+++	+++	++	+++	+++	+++
Chl'ramphenicol	30 µg	BioMerieux	-	+++	+++	++	+	+++	+++
Cloxacillin	25 µg	BioMerieux	-	+++	+	-	-	++	+++
Doxycycline	30 µg	BioMerieux	-	+++	++	-	-	+++	+++
Erythromycin	15 µg	Oxoid	-	+++	+	-	-	+	+
Gentamycin	10 µg	BioMerieux	++	+++	+++	++	+	+++	+++
Neomycin	30 µg	Oxoid	-	+++	++	-	+	-	++
Oxytetracycline	30 µg	Oxoid	-	+++	++	-	-	+++	+++
Penicilline G.	10 in	Oxoid	-	+++	-	-	-	-	++
Streptomycin	10 µg	Oxoid	-	-	+	-	-	-	-
Trisile sulph	300 µg	Difco	-	+++	+	-	+	++	+++

+++ = Inhibition zone 14 - 19 mm.

++ = Inhibition zone 8 - 12 mm.

+ = Inhibition zone 3 - 5 mm.

- = Insensitive.

\* = Cefosid (cefoperazone sod.) was tested 30 µg per disc.

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Tab. 3 : Therapeutic trial for some infected cows:

Nr. of cases	Isolated bacteria	Drug used	Route of injection	Dose/ duration
2	Klebsiella pneumoniae	Cefobid*	I/M*	500 mg /Q <sup>s</sup> / 5 days
	Corynebacterium bovis		I/V**	5 mg /kg / 5 days
2	Klebsiella pneumoniae		I/M	500 mg /Q/ 5 days
	Pseudomonas aeruginosa		I/V	5 mg /kg/ 5 days
	Corynebacterium bovis			
1	Enterobacter aerogenes		I/M	500 mg /Q/ 5 days
	E. coli (inactive)		I/V	5 mg /kg/ 5 days
	Staphylococcus aureus			
	Corynebacterium bovis			

# = Cefobid is a vial contain one gram of cefoperazone sodium

\* = I/M : Intramammary infusion

\*\* = I/V : Intravenous, calculated dose was dissolved in 500 ml. dextrose-saline and injected intravenously drop by drop.

S = Q : Infected quarter