INVESTIGATIONS ON BACTERIA CAUSING SUBCLINIAL MASTITIS IN CATTLE

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ABSTRACT

This research was carried on 80 quarter out of 600 quarter milk samples collected aseptically from 150 apparently healthy cows in 4 private farms at Alexandria Governorate (Abees – Ameria- Borg-El-Arab and Mariout ) and prove the affection of subclinical mastitis by use California mastitis test. The total incidence of subclinical mastitis in Alexandria Governorate was 13.33% .While the incidence in 4 farms (Abees – Ameria- Borg-El-Arab and Mariout ) were ( 14.3 %, 13.9% , 12.5%, and 10% ) respectively. The number of the bacterial isolates in these investigation was 92 isolates, while the number and frequency percentages of the isolates were Staphylococcus aureus 23 (25%), Streptococcus agalactia 20 (21.37%), Streptococcus dysagalactia 8 isolates (8.69%), E-coli 8 (8.69%), Arctenobacterium bovis 18 (19.26%), Pseudomonas aeruginosa 13 (14.13%), and Pseudomonas fluorescens 2 isolates (2.17%). Also, the sensitivity of the isolated organisms to antibiotics were discussed. It was found that most of the isolated species were highly sensitive to Chloramphenicol, Gentamycin and Cephaloxin and less sensitive to Tetracyclin and Streptomycin but were resistance to Penicillin and Amoxicillin. Public health significance was discussed.
INTRODUCTION

The most important disease affecting udder is mastitis which is a problem of considerable economic importance in dairy industry throughout the world. So the most mastitic cases occur in subclinical forms, hence the diseased animal continues, for a time to be a dangerous source of infection (Keisler, 1981). The subclinical mastitic milk may have a public health importance, since it may be due to a human pathogen which will cause an infection to the consumers of raw or inadequately heated milk (Wilesmith et al., 1986 and Sedeek et al., 2000). The subclinical mastitis constitutes a herd problem and the loss is nearly three times that of the clinical form. More attention had been focused for the diagnosis of subclinical mastitis (Joshi et al., 1976). Ghazi and Nair (2006) mentioned that the subclinical mastitis always characterized by the presence of germs, only, there is a hyperleucocytosis and certain modifications of the chemical properties of milk. This disease is considered as multifactorial where development of infection depends on presence of mastitic pathogens and a series of additional factors that act concomitantly (Eman et al., 2006). The most predisposing factors causing a lower resistance of udder for bacterial invasion are the general healthy condition of animal, the milking machine and bad milking habits (Tawfik et al., 1984). The subclinical mastitis problem was studied by several authors in different countries, Narendra et al., (1982) in India, Hussein et al., (1984) in Pakistan, Aleksandrova (1986) in Bulgaria, Lopes et al., (1990) in Brazil and Controbi et al., (1992) in Argentina. In Egypt this problem was studied by different authors, (Aziz et al., 1975, Abdelkarim and El-ashmawy, 1979, Bakr, 1986, El-Bayomi and Mahmoud, 1987, Aki, 1988, Amal et al., 1990, Nawal et al., 1996, Seddek et al., 1999, Awad and Abeer, 2003).and Hamdy et al.,(2007).

The present work was aimed to investigate the presence of the disease in...
some farms in different localities at Alexandria Governorate, together with the identification of the bacterial causative agent. Moreover, due to the public health hazard of antibiotic resistance for microbial antibiotic sensitivity patterns for infections were evaluated.

MATERIAL AND METHODS

Collection of samples:

Milk samples were collected from farms in 4 different areas in Alexandria Governorat from dairy apparently healthy udder of cows. The udder was thoroughly washed with soap and water, then dried and swabbed with 70% alcohol. The first stream of milk was discarded, then about 10-15 ml of milk was collected from the 4 quarters of the udder into a sterile tube. All samples were subjected to California mastitis test (C.M.T) according to Schalm et al.(1971). Samples giving (+ +) and over were considered positive and were marked and transported immediately to the laboratory where they were kept in refrigerator until bacteriological examination.

Bacteriological examination:

The milk samples were centrifuged at 300 rpm for 15 minutes and a loopful from the sediment of the pre-incubated milk sample at 37c° over night, was streaked on nutrient agar, blood agar and MacConkeys agar plates. The inoculated plates were incubated at 37c° for 48 hours and examined for bacterial growth. Isolated colonies were picked up and subcultured on the appropriate media for purification, studying the cultural characteristics, and identification by microscopic examination and biochemical reactions according to Cruickshank et al .(1975) and Koneman et al.(1988).

En-vetro antibiotic sensitivity test:

The disc and the agar diffusion method described by Cruickshank et al .(1975) was performed. Aportion of a single bacterial colony was selected and inoculated into 4 ml trypticase broth and incubated at 37 c°
for 18 hours. The culture was flooded on to the surface of well dried Muller – Hinton agar plates. The plates were tipped and excess of fluid was removed with a pipette, after being sure that entire surface was covered with the inoculum. The bacteria were allowed to settle for 15 minutes at 37 c°. The antibiotic discs were aseptically overlaid. The plates were allowed to stand for 30 minutes at room temperature after application of the discs, then after over night incubation at 37c°, the plates were examined for inhibition zones. The interpretation of results were carried out according to Finegold and Martine (1992).

RESULTS

Detection of subclinical mastitis:

As shown in Table (1) and (2) the results of examination of milk samples collected from 600 quarters of 150 apparently healthy cows for mastitis using California mastitis test revealed that 80 milk samples (13.33%) were positive for California mastitis test and were considered as subclinical mastitic cases while 520 milk samples (86.66) were normal cases.

Bacteriological findings:

As shown in Table (3) 80 subclinical mastitic milk samples were examined bacteriologically, all of them were positive for bacterial growth in an incidence of 100%. 7 different types of microorganisms were isolated and identified as Staphylococcus aureus 23 isolates (25%), Streptococcus agalactia 20 isolates (21.73%), Streptococcus dysagalactia 8 isolates (8.69%), E-coli 8 isolates (8.69%), Arctenobacterium bovis 18 isolates (19.26%), Pseudomonas aeruginosa 13 isolates (14.13%) and Pseudomonas fluorescens 2 isolates (2.17%). The distribution of the isolated bacteria causing subclinical mastitis in different 4 localities examined in Alexandria Governorate were illustrated in Table (2).
In vitro sensitivity test:

As shown in Table(4) 70 isolates of 7 different types of bacteria species isolated from the milk of subclinical mastitic cows were tested for their sensitivity to 9 different antimicrobial agents. The most effective antibiotics were Chloramphinicol, Gentamycin and Cephaloxin and followed by Tetracyclin and Stryptomycin which were less effective but they showed high resistance to Pencillin and Amoxicillin.

Table (1): Incidence of subclinical mastitis.

<table>
<thead>
<tr>
<th>Total No. of examined animals</th>
<th>Total No. of examined quarter milk samples</th>
<th>Apparently healthy quarter samples</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Subclinical cases</td>
</tr>
<tr>
<td></td>
<td></td>
<td>No.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>No.</td>
</tr>
<tr>
<td>150</td>
<td>600</td>
<td>80</td>
</tr>
</tbody>
</table>

Table (2): Incidence of subclinical mastitic cows in The examined farms.

<table>
<thead>
<tr>
<th>Farm</th>
<th>Number of examined quarter</th>
<th>Positive cases of examined quarter milk samples by C.M.T.</th>
<th>Percentages</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abees</td>
<td>260</td>
<td>37</td>
<td>14.3</td>
</tr>
<tr>
<td>El-Amreia</td>
<td>180</td>
<td>25</td>
<td>13.9</td>
</tr>
<tr>
<td>Borg El-Arab</td>
<td>80</td>
<td>10</td>
<td>12.5</td>
</tr>
<tr>
<td>Mariout</td>
<td>80</td>
<td>8</td>
<td>10.0</td>
</tr>
<tr>
<td>Total</td>
<td>600</td>
<td>80</td>
<td>13.33</td>
</tr>
</tbody>
</table>

C M T = California mastitis test.
DISCUSSION

Several methods have been reported for the detection of mastitis. The isolation of the causative micro-organisms is the most accurate, yet it is expensive and time consuming. Therefore, the need for a simple, quite, sensitive, rapid and reliable test sufficient to be applied on a large scale of animals is highly required. California mastitis test was recommended as a preliminary screening test for detection of subclinical mastitis (Dedie and Kieliverin 1960). This test indicator is one of the most reliable tests in diagnosis of subclinical mastitis in diary herds. However, the test is used to diagnose subclinical mastitis in the field, but to clarify the causative organism and for better treatment based on the sensitivity test bacteriological diagnosis is required. From the obtained data the incidence percentages of subclinical mastitis in 4 farms of different localities in Alexandria Governorate (Abees –El- Ameria-Borg-El-Arab and Mariout) were 14.3%, 13.9%, 12.5%, and 10% respectively (Table 2) with a total incidence (13.33%). The incidence is much less than that obtained by Chanders and Baxi (1975), Tsnoev et al. (1976), Glazer (1977), Verma (1978), Abdelkarim and El-Ashmawy (1979) and Ibtisam et al. (1993) who found that subclinical mastitis between dairy cows was 43.6%, 56%, 31.8%, 45%, 60% and 56.43% respectively. This lower incidence of subclinical mastitis in the present work reflects the good hygienic measures of examined, well fed animals and good immune status of them. On the other side the result is nearly similar to that recorded by Brodauf (1965) 18% and Seddek et al. (1999) 11.38%. On the other hand several authors failed to record any cases of subclinical mastitis in dairy cows, this indicates that the hygienic measures applied were very restricted and well controlled (Heever et al. 1976, Misra, 1976, Sharma et al., 1977, Klastrup and Halliwell 1977, Kafrelsheikh Vet. Med. J. Vol. 5 No. 2 (2007)
Investigations On Bacteria Causing Subclinical ... and Abd El-Halim, 1979). Besides, the difference of incidence percentages may be due to variation in enviromental conditions and food programme followed in these farms specially if the ration is mixed with selenium and vitamine E supply which improve the udder healthy condition (weiss et al., 1990). The results of bacteriological examination of 80 subclinical mastitic milk samples showed that all samples were positive for bacterial growth in an incidence of 100% (Table 3). This finding nearly coincides with the observation obtained by Verma et al.(1978), Anderson et al. (1982) and Seddek et al.(1999). From the mentioned data in Table(3) it was found that the main causative bacterial agents responsible for subclinical mastitis in dairy cows were Staphylococcus aureus, Streptococcus agalactia, streptococcus dysagalactia, E-coli ,Arctenobacterium bovis and Pseudomonas aeruginosa as well as 12 cases of mixed infection mostly Staphylococcus aureus, E-coli and Arctenobacterium bovis. Similar causative agents were previously described by Jain (1979) Abd El-All (1989) and Seddek et al.(1999). In this investigation the numbers and frequency of percentages of the isolates were as such Staphylococcus aureus (29.34%), Streptococcus agalactia (21.73%), Streptococcus dysagalactia (8.69%)E-coli (8.69%), Arctenobacterium bovis (19.26%), Pseudomonas aeruginosa(14.13%) and Pseudomonas fluorescens (2.17%). In this respect the present results are nearly similar to those obtained by different authors, Amal ,et al.(1990), Controbi, et al.(1992) ,Jha, et al.(1994), Aydin, et al.(1995) and Seddek, et al. (2000) who isolated different organisms which included Staphylococcus aureus (28.57%), E-coli (20.5%), Streptococcus agalactia(11.76%),Streptococcus dysagalactia (6.3%),Arctenobacterium bovis(9.8%) and Pseudomonas aeruginosa(15.6%) respectively. A higher incidence was obtained by El-Bayomi and Mahmoud (1987) and Morselt et al.(1995) who reported that Streptococcus agalactia and
Staphylococcus aureus were the predominant organisms with an incidence of (55.29%) and (65%) respectively. Also Rottschedt (1994) recorded that the main causative agent of subclinical mastitis in cows were Streptococcus agalactia and Staphylococcus aureus with a higher incidence (94.4%) and (58.8%) respectively, while Wilson et al. (1996) stated that the main causative bacterial agents were Staphylococcus coagulase negative, E-coli, Streptococcus agalactia and Streptococcus dysagalactia with an incidence of 21%, 40%, 63% and 48% respectively. It should be mentioned that the high incidence of the isolated bacteria causing subclinical mastitis reported by several authors may be attributed to the heavy contamination of bedding, housing, food materials, water, air and equipments which affect the healthy condition of animals and udder (Zecconi et al. 1994, Roberson et al. 1994 and Leibisch et al. 1994). Mixed infection had been observed in this study mainly Staphylococcus aureus with E-coli denoting the complexity of the disease, this is in agreement with Jain (1979) who stated that the Staphylococcus aureus may predispose the herd to infection by coliforms and other pathogens.

As regards the sensitivity test of the bacterial isolates, the obtained results showed that Chloramphenicol, Gentamycin and Cephaloxin were highly sensitive agents against most of the strains, while Tetracyclin and Stryptomycin were the less effective. On the other hand, most of the strains were resistant to Pencilllin and Amoxicillin. The previously mentioned results are supported by several authors (Jha et al.,1994, Aydinet al.,1995 and Nawal et al., 1996).

In conclusion, the subclinical mastitis in dairy cows is one of the most important serious disease due to public health importance, since Streptococci, E-coli and Staphylococci which are pathogenic for human
being are excreted in milk and would give rise to scarlet fever, septic sore throat, pyogenic infection and food poisoning to those consuming raw milk. Therefore good management practices such as milker's hygiene, sanitization of milking machine as well as controlling the predisposing factors should be considered among the major prophylactic measures to minimize the occurrence of the disease. Identification of the causative organisms and its sensitivity testing are important measures for the treatment and prevention of the disease.

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قسام البكتريولوجي / معهد بحوث صحة الحيوان – الإسكندرية

شمل البحث عدد 80 عينة لبن من 80 ربع لمقلابل مصاب بالتهاب الضرع تحت إكلينيكي عن طريق الكشف السريع بإختبار كاليفورنيا وذلك من بين 600عينة لبن من 600ربع لعدد 150 بقرة حليب في أربعة مناطق مختلفة بمحافظة الإسكندرية وهي مناطق أبيس، العامرية، برج العرب، وقطار مريوط الزراعي. و كانت نسبة الإصابة الكلية بالتهاب الضرع الخفي 13.3% حيث كانت نسبة الإصابة في مزارع مبركة أبيس 14.3% و العامرية 13.9% و برج العرب 12.5% و كانت الإصابة في مزارع قطار مريوط الزراعي 10.0%. و لقد أوضحت النتائج عزل 92 عزلة بكتيرية حيث تم عزل عدد 23 عزلة من الميكروب العنقودي الذهبي بنسبة (21.37%) و عدد 20 عزلة من الميكروپ السبحى الإجلاكتيا بنسبة (8.69%) و عدد 8 عزلات من الميكروب الولرونى بنسبة (19.2%)، كما تم عزل عدد 13 عزلة من ميكروب السيدموناس إفروجينوزا بنسبة (14.13%) و أخرى تم عزل عدد 2 عزلة من بكتيريا السيدموناس فلورسنس بنسبة (2.17%). و لقد تم إجراء اختبار الحساسية لهذه العوامل البكتيرية لعدد من المضادات الحيوية المختلفة و كانت معظم العوامل البكتيرية شديدة الحساسية لكل من الكليورامفينيكول و الجنتاميسين و السيفالوكسين و كانت قليلة الحساسية لكل من التراميسيلين و الإستريوميسين. كانت جميع العوامل المعزولة مقاومة للبسيلين و الأموكسيسيلين.

و قد تم مناقشة الخطورة الصحية لهذه الميكروبات المرضية على الصحة العامة وكذلك طرق الحد من إنتشارها.