Isolation and Characterization of Fungi from Clinical Bovine Mastitis

Mohammad Nazar Faisal, D. Rathnamma, S. Isloor, B.M. Veeregowda, R. Sharada and N.B. Shridhar

Department of Veterinary Microbiology, Veterinary College, KVAFSU, Hebbal, Bangalore- 560 024.

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Bovine mastitis is a disease caused by variety of microorganisms that incur huge economic losses to the dairy industry by decreasing milk production and increased costs of medication and culling. Bacteria have been recognized as the primary causative agents of mastitis in cattle. But, the role of fungi in causing mastitis in cows has gained importance only in recent years. Mycotic mastitis had been documented to be caused by various genera of yeasts. However, the most frequently encountered species are Candida spp, Trichosporon spp, Cryptococcus spp, Saccharomyces spp. and Aspergillus spp. The present communication reports the incidence of mycotic mastitis in dairy cows in Bangalore, Karnataka.

Materials and Methods

78 milk samples were collected from cows affected with clinical, recurrent mastitis and treated with various antibiotics. The milk samples were collected aseptically and each sample was inoculated on duplicate Sabouraud’s dextrose agar (SDA) plates containing chloramphenicol 400 mg/liter. One plate was incubated at room temperature (25 to 30°C) and other at 37°C and observed daily for fungal growth up to 30 days before considering the samples as negative. From plates showing fungal growth, individual colonies were picked up and sub cultured on SDA slants. The slants were stored at 4°C for further use. Molds were identified on the basis of colony characteristics and microscopic morphological features in Lactophenol cotton blue (LPCB) mounts and slide culture technique as recommended by Ajello et al. (1966). The yeasts and yeast-like fungi were primarily indentified on the basis of microscopic morphology in LPCB wet mounts and Gram’s staining. The genera and species of the yeasts were indentified on the basis of colony characteristics, growth characteristics on different media like corn meal agar with and without Tween - 80 , Sabouraud’s dextrose agar, urea hydrolysis, sugar fermentation, chlamydospore production on corn meal agar, Germ tube test Cambell and Sterwart, 1980). Fungal cultures were also identified at Agarkar Research Institute, Pune, (National Fungal culture Collection Centre).

Results and Discussion

Out of 78 cows affected with recurrent clinical mastitis, 20 cows were positive for fungal cultures. In the present study, the incidence of mycotic mastitis was 25.64 per cent in mastitic cows. Variable reports on the incidence of fungal mastitis in bovines have been reported earlier (Sharma et al.,1977 ; Sarma et al., 1980; Singh et al., 1992; Daljeet et al., 1996). The variability of mycotic mastitis could be due to variation in the climate, treatment and management condition of the place.

Twenty nine different fungi were isolated from 20 clinical mastitis cases (Table I). The common yeasts isolates were Candida parapsilosis (6), Candida tropicalis (3), Trichosporon coremiiforme (2), Rhodotorula spp (2), Cryptococcus magnus (1), Debaromyces nepalensis (1), Sporisorium formosanum (1), and unidentified yeast (1). Molds isolated were Aspergillus ochraceus (3), Cladosporium spp (2), Penicillium spp (2), non-sporulating dematiaceous fungi (2), Filamentous yeast group(l) and unidentified mold (2).

The use and abuse of antibacterial drugs, treatment with contaminated antibiotic solutions, as well as syringe or other materials brought in contact with the mammary gland.
may favour yeast colonization in cow’s udder
(Carolina et al., 2011). The use of antimicrobials
for a long period is the main factor that precipi-
tates the occurrence of mycotic mastitis because
they affect the microflora of the mammary
glands, which acts as an animal’s natural
defence. Many outbreaks of mycotic mastitis
have occurred following antibiotic therapy. The
percentual occurrence of the yeasts was higher
than moulds in the present study with isolation
of 17 yeasts (58.62 per cent) and 12 moulds (41.37
per cent). The common occurrence of yeasts,
when compared to moulds, might be due to their
better perpetuation in the mammary gland and
also the fact that some genera of yeasts can
utilize antibiotics like penicillin or tetracycline
as a nitrogen source for their growth as reported
by Menhnert et al. (1964).

Yeasts like Candida parapsilosis, Candida tropicalis, Rhodotorula, Cryptococcus and Trichosporon were also isolated from bovine milk samples by Monga and Kalra (1971), Jan and Dhillon (1975), and the moulds such as Aspergillus ochraceus (25%), Cladosporium spp
(16.66%), Penicillium spp (16.66%) have also
been isolated from cases of subclinical and clinical bovine mastitis (Ginnis et al., 1982), Sharma et al. (loc.cit), Singh et al., (loc.cit), Costa et al.,
(loc.cit). Daljeet et al.,(loc.cit). The isolation of
fungi post-antibiotic treatment suggest that they are not only reactant to them but produce
disease in the absence of fast multiplying bacte-
rial competition by utilizing antibiotics for their
basic growth requirement (Mehnert et al., loc.
cit).

The higher incidence of yeast infection
over moulds may be attributed to its ability to
produce and propagate well in the udder tissue
just like bacteria (Aisworth and Austwick, 1959).

Summary
Out of 78 milk samples obtained from clinical
mastitis cases, 20 samples (25.64 per cent) were
positive for fungal agents with 29 fungal isolates.
Out of 29 fungal isolates, 17 were yeasts and 12
were molds.

Table I. Fungal isolates recovered from bovine mastitis cases

<table>
<thead>
<tr>
<th>Fungal isolates</th>
<th>No. of isolates</th>
<th>Per cent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yeasts</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Candida parapsilosis</td>
<td>6</td>
<td>35.29</td>
</tr>
<tr>
<td>Candida tropicalis</td>
<td>3</td>
<td>17.64</td>
</tr>
<tr>
<td>Trichosporon spp., close to T. coremiiforme</td>
<td>2</td>
<td>11.76</td>
</tr>
<tr>
<td>Rhodotorula spp.</td>
<td>2</td>
<td>11.76</td>
</tr>
<tr>
<td>Cryptococcus magnus</td>
<td>1</td>
<td>5.88</td>
</tr>
<tr>
<td>Debaromyces spp., close to D. nepalensis</td>
<td>1</td>
<td>5.88</td>
</tr>
<tr>
<td>Sporisorium formosanum</td>
<td>1</td>
<td>5.88</td>
</tr>
<tr>
<td>Unidentified yeasts</td>
<td>1</td>
<td>5.88</td>
</tr>
<tr>
<td><strong>Total yeast isolates</strong></td>
<td><strong>17</strong></td>
<td><strong>58.62</strong></td>
</tr>
<tr>
<td>Molds</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aspergillus ochraceus</td>
<td>3</td>
<td>25.00</td>
</tr>
<tr>
<td>Cladosporium spp.</td>
<td>2</td>
<td>16.66</td>
</tr>
<tr>
<td>Penicillium spp.</td>
<td>2</td>
<td>16.66</td>
</tr>
<tr>
<td>Yeast group + Non sporulating dematiaceous</td>
<td>2</td>
<td>16.66</td>
</tr>
<tr>
<td>Filamentous yeast group</td>
<td>1</td>
<td>8.33</td>
</tr>
<tr>
<td>Unidentified moulds</td>
<td>2</td>
<td>16.66</td>
</tr>
<tr>
<td><strong>Total mold isolates</strong></td>
<td><strong>12</strong></td>
<td><strong>41.37</strong></td>
</tr>
<tr>
<td><strong>Total fungal isolates</strong></td>
<td><strong>29</strong></td>
<td></td>
</tr>
</tbody>
</table>
Effect of Addition of Cysteine Hydrochloride on the Liquid Storage (5°C) of Mithun (Bos frontalis) Semen

P. Perumal1, Kezhavituo Vupru and C. Rajkhowa
National Research Centre on Mithun (ICAR), Jharnapani, Nagaland – 797 106

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Mithun (Bos frontalis) is a semi-wild free-range, has an important place in the social, cultural, religious and economic life of the tribal population in North-Eastern hill region of India. Since mithuns are semi-wild animals and not fully domesticated, natural breeding is practiced in this species with accomplished limitations like cost and disease transmission. The use of artificial insemination for improvement of its pedigree is becoming more essential. Mithun semen normally contains anti-oxidants than can offset lipid peroxidation. But the concentration of these antioxidants is reduced during dilution and storage that affects the semen quality during storage. Thiol such as cysteine hydrochloride and glutathione (GSH) prevents the loss of sperm motility in liquid storage (Uysal and Bucak, 2007) and in frozen-thawed bull semen (Perumal et al., 2011). Cysteine is a precursor for intracellular glutathione biosynthesis and increases the GSH level. The objective of this study was to assess the effect of cysteine Gxdurochloride on the seminal parameters of mithun semen to pursue future sperm

1Corresponding author: Email : perumalponraj@gmail.com