Diagnostic and Therapeutic Studies on Mycotic Mastitis in Cattle

Esraa M. Bakr, Abd El-kareem M. Abd El-Tawab, Tharwat M. Elshemey, Amir H. Abd-Elrhman
Department of Animal Medicine, Faculty of Veterinary Medicine, Alexandria University

Key words: PCR, candida, Aspergillus, Mycotic Mastitis

ABSTRACT:

40 milk samples were collected from cattle suffering from chronic mastitis with no or poor response to therapy with common antibiotics, these samples were subjected to bacteriological and mycological culture then confirmed by traditional fungal confirmatory procedures and multiplex PCR (m-PCR). The results showed 37 positive samples for fungal culture hence Candida albicans took the lead of them by 60 %, Aspergillus fumigatus 22.5%, Aspergillus niger, 10 % while Cryptococcus neoformans not detected in any sample. Mixed bacterial and fungal infection was detected in 83.78% while fungi as a sole etiological agent were detected in 16.22% of samples. The PCR succeeded to distinguish between Aspergillus fumigatus, Aspergillus niger and Candida albicans more accurate and fast than traditional confirmatory methods. Pure mycotic mastitis cases were subjected to treatment by a new novel drug consisting of extract of medicinal plants (garlic and sesame oil). All treated cases responded well to this drug and returned to normal udder condition and to full milk production.

1. INTRODUCTION

Bovine mastitis is a disease caused by a wide variety of microorganisms that causes large economical loses and damages to the dairy industry by decreasing milk production and through increasing costs of antibiotic treatment and culling (Zaragoza et al., 2011).

Bovine mycotic mastitis is usually caused by yeasts, but mastitis due to filamentous fungi mostly aspergillus fumigatus has been reported it occurs as sporadic cases affecting a small percentage of cows or as outbreaks affecting the majority of animals (Abd El Razik et. al. 2011).

The incidence of mycotic mastitis is on the rise and the most frequently isolated fungi from milk are cryptococcus neoformans and candida albicans due to prolonged and indiscriminate use of antibiotic and steroids in intramammary therapy for mastitis as well as prevalence of fungal organisms on dairy farms. (Rayaz Ahmed et al. 2013).

Candida spp. was included in mycotic cases of bovine mastitis especially candida glabrata and candida kruse. On the other hand, samples from the subclinical mastitis group showed a diversity of candida species, including C. zeylanoides, C. norvegica, C. viswanathii, C. guilliermondii and candida tropica (Zaragoza et al. 2011).

The early, rapid and accurate identification of the pathogenic fungus is critical for timely, appropriate management. The conventional identification of pathogenic fungi in the clinical microbiology laboratory is based on morphological and physiological tests often require 3 or more days and may be inaccurate. In recent years a multiplex PCR method was developed to identify simultaneously multiple fungal pathogens in a single reaction (Luo and Mitchell 2002).

Antifungal drug resistance of aspergillus might partially account for treatment failure. Resistance of Candida species to drugs has led scientists to pay more attention to traditional medicine herbs. Due to the limitations in the treatment of fungal diseases such as shortages, high prices, antifungal side effects and drug resistance or reduced susceptibility to fungal drugs we decided to study the antifungal effects of herbal extracts of Syzygiumaromaticum and Punicagranatum (Mansourian et al. 2014).

Laboratories have demonstrated impressive in vitro MICs using allitridium, one of the garlic oil derivatives against arrange of medically important fungi. In addition it has been demonstrated that allitridium shows in vitro synergy with amphotericin B one of the main i.v. antifungal agents (Davis 2005).

Aim of the work is the determination of the mycotic agents causing bovine mastitis,
improvement of confirmatory methods from isolation of mycotic agents by using PCR and implementation of new drugs for treatment of mycotic mastitis.

2. MATERIAL AND METHODS

2.1. Clinical examination
Clinical examination was carried out according to (Radostatis et. al. 2007). Abnormalities of size and consistency were assessed by palpation and inspection of the udder to detect fibrosis, inflammatory swelling or atrophy of mammary tissues. The udder was viewed from behind and the two back quarters were examined for symmetry before and after milking. Whole quarters were palpated including the region of milk cistern. The supramammary lymph node and the teats. The animal was examined for systemic reactions including fever, general depression and anorexia.

2.2. Sample collection
The udder, teat orifice and hands of the milkers were perfectly cleaned with water and soap and disinfected with 70% ethyl alcohol before collection of milk samples. The first streams of milk were discarded, the second streams of milk were collected in clean sterile capped bottle, each bottle was given a serial number. Samples were refrigerated 4°C during transportation to the laboratory and examined mycologically and bacteriologically as soon as possible (Radostatis et. al. 2007).

2.3. Mycological examination
Samples from cows with a typical symptom of clinical mastitis incubated aerobically at 37 °C for 18-24 hrs then centrifuged, then the cream, supernatant fluid were discarded, the sediment was streaked on Nutrient agar, MacConkey agar and blood agar. Biochemical identification of isolated bacteria: according to Quinn et.al. (2002) Catalase test, Tube Coagulase test, Indole production test: Kovac’s (1928).

2.4. Bacteriological examination
Samples from cows with a typical symptom of clinical mastitis incubated aerobically at 37 °C for 18-24 hrs then centrifuged, then the cream, supernatant fluid were discarded, the sediment was streaked on Nutrient agar, MacConkey agar and blood agar. Biochemical identification of isolated bacteria: according to Quinn et.al. (2002) Catalase test, Tube Coagulase test, Indole production test: Kovac’s (1928).

2.5. Detection of yeast and fungi by mPCR
Oligonucleotide primers used in mPCR: Luo and Mitchell (2002): They have specific sequence and amplify a specific product as shown in Table (1).

Source: South MC Dowell Blvd, Petaluma USA.

2.5.1. DNA extraction:
For molecular studies a lapful of fungal colony 1 mm in diameter from Sabouraud dextrose agar plate was suspended in 500 µl of saline in eppendorf tube then vortexed and centrifuged at 15000 xg/ 10 minutes, the supernatant was discarded then the sediment was resuspended in 500 µl of sterile distilled water then the mixture of sediment and distilled water was vortexed and boiled for 30 minutes then centrifuged at 15000 xg for 10 minutes and finally 0 µl of supernatant was used as a DNA template.

2.5.2. Preparation of PCR Master Mix: Emerald Amp GT PCR master mix (Takara) code No. RR310A Contains: Emerald Amp GT PCR master mix (2x premix), PCR grade water. a 25 µl of master mix was prepared by adding 12.5 µl of Emerald Amp GT PCR master mix (2x premix), 4.5 µlPCR grade water, .1 µl forward primer(20pmol), 1 µl reverse primer (20pmol) and 6 µl template DNA.

### Table 1. A. fumigatus, C. albicans and Cryptococcus neoformans – specific primer pairs characteristics.

<table>
<thead>
<tr>
<th>Organism</th>
<th>Primer name</th>
<th>Sequence (5’→3’)</th>
<th>Amplicon size (bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aspergillus fumigatus</td>
<td>AspFuF-32</td>
<td>CGC CGA AGA CCC CAA CAT GAA CGC</td>
<td>≈ 385</td>
</tr>
<tr>
<td></td>
<td>AspFuR-32</td>
<td>TAA AGT TGG GTG TCG GCT GGC</td>
<td></td>
</tr>
<tr>
<td>Candida albicans</td>
<td>CandF-32</td>
<td>TTT ATC AAC TTG TCA CAC CAG A</td>
<td>≈ 273</td>
</tr>
<tr>
<td></td>
<td>CandR-32</td>
<td>ATC CCG CCT TAC CAC TAC CG</td>
<td></td>
</tr>
<tr>
<td>Cryptococcus</td>
<td>CryF-32</td>
<td>GAA GGG CAT GCC TGT TTG AGA G</td>
<td>≈ 136</td>
</tr>
</tbody>
</table>
2.5.2. DNA extraction:
For molecular studies a loopful of fungal colony 1 mm in diameter from Sabouraud dextrose agar plate was suspended in 500 µl of saline in epindroff tube then vortexed and centrifuged at 15000 xg/ 10 minute, the supernatant was discarded then the sediment was resuspended in 500 µl of sterile distilled water then the mixture of sediment and distilled water was vortexed and boiled for 30 minutes then centrifuged at 15000 xg for 10 minutes and finally 0 µl of supernatant was used as a DNA template.

2.5.2. Preparation of PCR Master Mix: Emerald Amp GT PCR master mix (Takara) code No.RR310A Contains: Emerald Amp GT PCR master mix (2x premix), PCR grade water. a 25 µl of master mix was prepared by adding 12.5 µl of Emerald Amp GT PCR master mix (2x premix), 4.5 µl PCR grade water ,1 µl forward primer(20pmol),1 µl reverse primer (20pmol) and 6 µl template DNA.

2.5.3. Cycling condition of the primers during PCR:
PCR amplification condition were 5 minutes of denaturation at 96 °C ,followed by 40 cycles of 94°C for 30 seconds, 58 °C for 30 seconds , 72°C for 30 seconds and a final extension step of 72 °C for 15 minute .

2.5.4. DNA Molecular weight marker: Gel Pilot 100 bp. Ladder (cat.no. 239035) supplied from QIAGEN (USA), Number of bands: 5 size range 100-600 bp. The ladder was mixed gently by pipetting up and down. 6 µl of the required ladder were directly loaded.

2.5.5. Agarose gel electrophoreses: It was done according to (Sambrook et.al., 1989).

2.6. Treatment trials of fungal mastitis cases.
6 cases of confirmed fungal mastitis as a sole etiological agent subjected to new drug (alternative therapy) represented as a blend of two aromatic oil(garlic oil extract and sesame oil extract (El captain pharm) by ratio 3:2 respectively for 7 days first dose given i/v then completed by i/m route. The animals were observed during treatment period and the results of treatment were recorded day after day during treatment.

3. RESULTS:
3.1. Clinical picture of fungal mastitis:
The examined animal showed chronic form of yeast mastitis which Characterized by hardness of udder, reduction in milk yield with watery milk and flakes were noticed. Chronic mastitis caused by mold distinct by hardness of udder and reduction in milk yield with straw color milk viscid in consistency.

3.2. Percentage of fungal mastitis among cattle by culture:
Out of 40 cases of chronic mastitis which did not respond to antibiotic therapy 37 cases were positive for mycological culture represented 92.5 %, the isolated fungi were C. albicans 60 %, Aspergillus fumigatus 22.5%, Aspergillus niger10% to shown in table (2).

<table>
<thead>
<tr>
<th>Organism</th>
<th>No. of samples</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A.fumigatus</td>
<td>9</td>
<td>22.5 %</td>
</tr>
<tr>
<td>C.albicans</td>
<td>24</td>
<td>60 %</td>
</tr>
<tr>
<td>Cryptococcus</td>
<td>Nil</td>
<td>0%</td>
</tr>
<tr>
<td>neoformans</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A.niger</td>
<td>4</td>
<td>10 %</td>
</tr>
</tbody>
</table>

Table 2. Number and percentage of mycotic infection detected by culture:

<table>
<thead>
<tr>
<th>Organism</th>
<th>No. of samples</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A.fumigatus</td>
<td>9</td>
<td>22.5 %</td>
</tr>
<tr>
<td>C.albicans</td>
<td>24</td>
<td>60 %</td>
</tr>
<tr>
<td>Cryptococcus</td>
<td>Nil</td>
<td>0%</td>
</tr>
<tr>
<td>neoformans</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 3. Number and percentage of mycotic infection detected by PCR
3.3. Results of multiplex PCR applied on fungal and yeast culture using primers of A. fumigatus.

Lane 1: 100 bp Ladder.
Lane 2, 3, 4, 6: *Aspergillus fumigatus* positive samples.
Lane 5, 7: Negative samples for *A. fumigatus, candida albicans* and *Cryptococcus neoformans*.

![Image of PCR gel with bands](image)

3.4. Percentage of mycotic mixed with bacterial infection.

Table (4) explained that number and percentage of fungi isolated hence *C. albicans* take the lead of them (74.2%) followed by A. fumigatus (16.13 %) and A. niger (9.67 %). The table showed different type of bacteria associated with previously fungi responsible for mastitis in this group. Hence *A. fumigatus* and *Salmonella* represented (9.68%), *A.fumigatus* and *Staphylococcus spp.* (6.45%), *Candida albicans* and *Staphylococcus spp.* (9.68%), *C.albicans & streptococcus spp.* (16.13%), *C. albicans & Salmonella* (25.81%), *C. albicans & E.Coli* (22.58%), *A. Niger & E.Coli* (6.45%) and *A. Niger & Staphylococcus spp.* (3.22%).

<table>
<thead>
<tr>
<th>Organism</th>
<th>No</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>A.fumigatus &amp; <em>Salmonella</em></td>
<td>3</td>
<td>9.68</td>
</tr>
<tr>
<td>A.fumigatus &amp; <em>Staphylococcus spp.</em></td>
<td>2</td>
<td>6.45</td>
</tr>
<tr>
<td>C.albicans &amp; <em>Staphylococcus spp.</em></td>
<td>3</td>
<td>9.68</td>
</tr>
<tr>
<td>C. albicans &amp; <em>Streptococcus spp.</em></td>
<td>5</td>
<td>16.13</td>
</tr>
<tr>
<td>C.albicans &amp; <em>Salmonella</em></td>
<td>8</td>
<td>25.81</td>
</tr>
<tr>
<td>C.albicans &amp; <em>E.coli</em></td>
<td>7</td>
<td>22.58</td>
</tr>
<tr>
<td>A.Niger &amp; <em>E.coli</em></td>
<td>2</td>
<td>6.45</td>
</tr>
<tr>
<td>A.Niger &amp; <em>Staphylococcus spp.</em></td>
<td>1</td>
<td>3.22</td>
</tr>
</tbody>
</table>

3.5. The percentage of fungi among animals which has completely no response to antibiotic therapy:

This group of cows precisely had no response to antibiotic therapy after long duration extended for several months or even short duration few days. Its culture revealed that fungi was the sole etiological agent. As shown on table (5) which defined that *Aspergillus fumigatus* responsible for the majority of mastitic cases refractory to treatment represented (66.66%) in this study, *Candida albicans* (16.67%) and A. niger (16.67%). These result in our study referred to the percentage of yeast and mould as a sole etiological agent causing mastitis represented 16.22% among total positive result.

3.6. Treatment trials:

All cases that not responded completely to antibiotic therapy which fungi was the sole causative agent as shown in previously table (5) subjected to new drug treatment even in cases neglected for long time lasted months, this appear after first dose hence the milk changed to its normal color and sever hardness in quarter started to decay.

Table 5. Percentage of fungi among animals which has completely no response to antibiotic therapy.

<table>
<thead>
<tr>
<th>Organism</th>
<th>No</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>A.fumigatus</em></td>
<td>4</td>
<td>66.66%</td>
</tr>
<tr>
<td><em>C.albicans</em></td>
<td>1</td>
<td>16.67%</td>
</tr>
<tr>
<td><em>A.niger</em></td>
<td>1</td>
<td>16.67%</td>
</tr>
</tbody>
</table>
4. DISCUSSION: Mastitis is a most devastating disease condition in terms of economic losses occurring throughout the world. According to Zaragoza et al., A. fumigatus & Staphylococcus spp. 6.45%, A. fumigatus & Salmonella 9.68%. C. albicans was of economic losses occurring throughout the world. The results of this study showed that A. fumigatus & Staphylococcus spp. & etiological agents may vary from place to place. The ratio depending on climate, animal species and animal husbandry and include wide variety of gram positive and gram negative bacteria and fungi (Deb et. al. 2014). Staphylococcus spp. 3.22% this result stole our attention to combat this invasion by avoiding the numerous debates because the incriminated agents continuous and indiscriminate use of antibiotic or using often contaminants of the outside or the combination of antimycotic and antibacterial agents (Akdouch et al. 2014). Mycotic mastitis had been documented to be caused by various genera. Yeasts are single celled organisms that are ubiquitous in mounds and yeasts beside bacterial mastitis, the mastitis environment and they are considered opportunistic fungi frequently encountered fungi are Candida spp. which characterized by hardness of udder, Aspergillus spp., Trichosporon spp. & Cryptococcus spp. When natural defense mechanism is lowered, Saccharomyces spp., Penicillium spp. (Ghodasara et al. 2011 and Pengova 2002). Outbreaks caused by yeast have particularly been reported in intensively managed herds in which there were failures in environmental hygiene or in association dairy herds but sometimes it can occur in epizootic with repetitive intramammary treatment (Geraldo Costa et al. 2000). An important predisposing factor in farm animals especially considered an environmental mastitis as they are usual Aspergillus (Krukowski et al. 2000). The agent in cow’s mastitis (Suhyla and Seyhan 2010) multiplication of fungi during antibiotic therapy is important and considered opportunistic infection. Mastitis caused by yeast is increased and antibiotic treatment is an important predisposing factor in farm animals especially considered an environmental mastitis as they are usual Aspergillus (Krukowski et al. 2000). The agent in cow’s mastitis (Suhyla and Seyhan 2010) multiplication of fungi during antibiotic therapy is important. The results of this study showed that A. fumigatus & Staphylococcus spp. 6.45%, A. fumigatus 22.5%. A. Niger 10% mixed produced chronic mastitis characterized by hardness of udder, milk with exception of C. Krusei, C. Parapsilosis decreased amounts of vitamin A in the glandular epithelium and irritating factors of antibiotic Trichosporon capitatum (Awad et. al. 1980). (Wladyslaw et. al. 2010).

This study was applied on chronic cases of mastitis. In this study, the examined animal showed chronic from no or poor response to antibiotic therapy, in our study mastitis which characterized by hardness of udder, the total percentage of mycotic mastitis was 92.5%, reduction in milk yield with watery milk and flakes all examined samples, the most isolated organism were noticed. In case of mastitis where mold was C. albicans 60%, A. fumigatus 22.5%, A. Niger 10% mixed produced chronic mastitis characterized by hardness of udder, milk with exception of C. Krusei, C. Parapsilosis decreased amounts of vitamin A in the glandular epithelium and irritating factors of antibiotic Trichosporon capitatum (Awad et. al. 1980). (Wladyslaw et. al. 2010).

Mycotic mastitis are split into two big groups according to (Scaccabarozzi et al., 2003). Yeast and yeast like fungi to the moment of appearance, primary mycotic mastitis produced chronic mastitis in which hardness of udder, (bacterial preliminary mastitis) and secondary mycotic mastitis in milk yield with watery milk and flakes mastitis which appear often straightway without noticed (Sukumar and James 2012) . Rapid and antibiotic treatment (Akdouch et al. 2014). Mixed identification of mastitis pathogens is (fungal and bacterial) infection in our study represents an important for disease control. Bacterial culture and 83.78% from total positive mycological culture were identified is considered the gold standard in
mastitis diagnosis but is time consuming and results. Drug discovery strategies based on natural product and many culture negative samples (Keane et al., 2013). Traditional medicine are re-emerging as attractive option need not always be confined to new molecular entities. Deficiencies with current diagnostic approaches include: Tactically designed carefully standardized, synergistic poor specificity and poor sensitivity; further multidimensional, herbal formulation and botanical drug culture based phenotypic identification techniques product with robust scientific evidence can also be slow and especially prone to misidentification of fungal alternative (Patwardhan and Mashekar, 2009). Records pathogens. Molecular microbiological techniques highlight in herbal medicine is renaissance of medicinal plants and their therapeutic values appear specific only in classic literature of traditional knowledge in detection and identification of fungi from clinical cultures of the east and west but also in numerous specimens or cultures (Geoffrey et al., 2006). Recent publications and databases developed by several institutes and organization such as the university Illions In this study we used multiplex PCR as confirmatory test, Indian Institute of Ayurveda and method for diagnosis of mycotic mastitis directly from microorganisms and Mahidol university (Nopamart culture. From Table 2, Figure 1 we confirmed our result). In our study we used new herbal drug (formula), hence the use of multiplex PCR could to discriminate alternative medicine to avoid side effects of common between A. fumigatus, A. niger, C. albicans. Antifungal. This contains garlic oil extract (GOE) and Cryptococcus neoformans, as a matter of fact garlic, different brands and lots of odourless garlic and efficient and lack of accuracy regardless of biochemical representative samples of garlic oil, freeze dried garlic tests which is time consuming. This indicated that young and aged garlic exhibited an inhibitory effect on far the multiplex PCR providerapid, sensitive antifungal agent. Cytochrome p450 2c91, 2c19, 3A4, 3A5 and 3A7 specific technique for identification of fungal pathogens. Metabolism of a marker substrate (Brian et al., 2001). One of the most biologically active compounds of garlic is allicin (Diallylthiosulfinate or Because the toxin is excreted in cow's milk, however. Diallyldisulfide) (Londhe et al., 2011), its minimal is important for public health, aflatoxin is an important inhibitory concentration is 1.5-6.3 µg/ml (Nopamart consideration in the etiology of human hepatocellular carcinoma). It has demonstrated that Allifirudium shows in carcinoma (Masoud et al., 2012). The consumption of garlic oil with amphotericin B. one of the main i/v contaminated milk by fungi of the Candida spp. antibacterial agents (Davis 2005). Its extensive repertoire toxin produced by them may be harmful to human. Inhibitory constituents makes garlic an interesting health. Risks are always present since the product diabetes alternative to single-site-specific antibiotics or undergo any pasteurization and thus people especially synthetic organic compounds for combating C. sick ones may receive opportunistic mycosis (Ludmillicans. Anticandidal effects of garlic similarly include et al., 2011). It is noteworthy that the adverse effect worldwide range of ultra-structural lesions affecting among antifungals and among animal species have mitochondrial membranes, organelles and cytoskeletal been evident mostly in systemic administration azegization (Katey et al., 2009). Garlic oil and 2 of its could result in a general lack of applicable clinical organo sulfur components, diallyl disulfide and literature for antifungal use in a given animal species trisulfide (DATs) can effectively upregulate which leads to an extrapolation on the interpretation (GSH T) mRNA and protein expression. Glutathione S- use of minimal inhibitory concentration data transferase (GST) is a phase II enzyme that catalyzes addition different pharmacokinetic properties of the conjugation of glutathione with a variety of antifungal drugs across animal species as well as xenobiotic xenobiotics and facilitates their excretion pregnant and young animals may contribute to (Gibong et al., 2010). In this regard we use it for observed side effects, due to the fact that the majority treatment of mycotic mastitis in several cases among antifungal drugs extensively used in animal are special pregnant animals. These mastitic females approved for human use (Nopamart 2011). No are subjected to treatment by injection intravenously or formulations are available in the market for subcutaneous for 5 to 7 days mastitis treatment (Amsaveni 2012). Fungal drug successfully once daily. We recorded complete resistance is an acute issue due to the limited number of recovery for these cases. We confirmed that it is safe, antifungal compounds; alternative strategies utilizing combination therapy will become more attractive (Makhlouf et al., 2011).
environmental mastitis problems, maintenance of a clean, dry, comfortable environment, proper milking procedures, proper maintenance and use of milking equipment, good record keeping, appropriate management of clinical mastitis during lactation, effective dry cow management, maintenance of biosecurity for contagious pathogen and culling of chronically infected cows (Henk et al., 2011).

**Conclusion:** *C. albicans* and *Aspergillus* spp. are the main etiological agents of mycotic mastitis in Egypt. The use of antibiotics as the standard treatment for bovine mastitis makes the evaluation of fungi as a primary cause of this disease in addition to the high cost of human antifungal but also to replace contaminated syringes, cannulas, contaminated antibiotic preparation by yeasts and fungi extremely difficult. PCR is easy and accurate, sensitive specific technique for confirming of etiological agent of mycotic mastitis. Vigorous need for alternate herbal remedy for mycotic mastitis forced us to apply this field not only to avoid shortage, side effects and the possibility of a safe and effective antifungal prophylactic. Microbes. 48 (2):95-100.

**Materials and Methods**

**Results:** We applied new remedy from garlic oil extract against *Candida* species isolated from Anatolian buffaloes with mastitis in western turkey. Vet. Clin. Microbiol. 44 (3): 876–880.


**Conclusion:** We evaluated the antifungal properties, safety and anti-carcinogenic effect of garlic oil extract against *Candida* species isolated from Anatolian buffaloes with mastitis in western turkey. Vet. Clin. Microbiol. 44 (3): 876–880.

**5. REFERENCES**


Pachauri, S., Puneet, V., Sandeep, K. D., Manoj, K. G. 2013. Involvement of fungal species in bovine mastitis in and around Mathura, India vet. world. 393-395.


