

# Prevalence of yeasts in bovine mammary gland infections and teat cups of milking machines

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## 효모균에 의한 젖소 유방염과 유두컵내의 오염상태

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**초록** : 진주근교에서 효모균에 의한 젖소의 준임상형 유방염 발생상황과 이들균의 유두컵내의 오염상태를 조사하였던 결과는 다음과 같다.

총 검사분방 330개 중 12분방으로부터 효모균이 분리되어 이로인한 준임상형 유방염의 발생율은 3.6%이었다. 분리균 12주는 *Candida pseudotropicalis* 4주, *C. tropicalis* 3주, *C. krusei* 2주, *C. albicans* 2주 및 *Rhodotorula* spp 1주로 동정되었으며, 분리균의 91.7%가 *Candida*속 균이었다. 유두컵 200개 중 20.5%로부터 *C. pseudotropicalis* 13주, *C. guilliermondii* 9주, *C. tropicalis* 7주, *C. krusei* 5주, *C. parapsilosis* 5주, *C. albicans* 3주, *Torulopsis glabrata* 2주, *Geotrichum candidum* 2주 및 미동정 효모균 5주가 분리되었다.

**Key words**: cow, mastitis, candida, yeasts.

### Introduction

Yeast-induced mastitis has increased in recent years and it plays an important role in the economic loss due to repetitive mastitis problem in dairy cows.<sup>1-4</sup> Yeasts frequently contaminate bovine mammary glands as opportunistic pathogen by intramammary antibacterial preparations and instruments, skin of the udder and teats, milker's hands, milking machines, feed, water, soil, and sanitizing solutions.<sup>1,3,5,6</sup> These produce mastitis when defence mechanisms of the host are suppressed or competitive bacteria are destroyed by antibacterial drugs.<sup>7,8</sup>

In Korea, infections of bovine mammary glands by the species of *Candida*, *Cryptococcus*, *Torulopsis*, *Rhodotorula* and *Hansenula* were recognized.<sup>4,9,10</sup> And

further studies are necessary in order to establish the extent of the causative organism and infection source of yeast mastitis. In view of these problem, the present study was performed to determine the subclinical yeast infection in the mammary glands of dairy cows and yeast contamination in the teat cups of milking machines in Chinju, Korea.

### Materials and Methods

**Samples tested**: Milk samples were collected aseptically from 330 quarters of 161 dairy cows at the suburb of Chinju city from June to October 1987. At this time liquid contents remained in 200 teat cups of milking machines were also swabbed aseptically. These were brought back to the laboratory for immediate assay. Somatic cells in milk were coun-

ted by rolling ball viscometer (Model RAI, Division of Refrigeration Engineering Co, New Zealand). Milk samples showing somatic cell counts of more than 500,000 per ml were diagnosed as subclinical mastitis, and then subjected to the microbiological examination.

**Isolation of yeasts:** The milk and teat cup samples were plated onto Sabouraud's dextrose agar (SDA) containing penicillin (20 units/ml) and streptomycin (100 µg/ml).<sup>11</sup> After incubation for 24 to 72 hours at 37°C, isolated yeasts were maintained on SDA slant for identification. The milk samples were also cultured on 5% sheep blood supplemented trypticase soy agar to confirm mere bacterial mastitis, which was ruled out in result evaluation.

**Identification of yeasts:** Identification of the yeast isolates was based on the morphologic and biochemical characteristics described by Richard et al<sup>1</sup> and Larone.<sup>12</sup>

Dalmau mounts of all isolates cultured on cornmeal agar at 25°C were observed microscopically for mycelial growth and morphology. Carbohydrate fermentation test was carried out using dextrose, maltose, sucrose, lactose and galactose. Also included in the characterization were abilities of the isolates to grow on Sabouraud's dextrose broth at 37°C and SDA with cycloheximide at 25°C, to develop germ tube or capsule, and to hydrolyze urea at 25°C.

Serological identification was made with standard candida antiserum (Candida Check, Iatron Laboratories Inc., Japan) by the manufacturer's indication.

## Results

Incidence of yeast-induced subclinical mastitis in dairy cows at Chinju area was 3.6% of 330 quarters (7.5% of cows). From the infected quarters 12 strains of yeasts were isolated. Contamination rate of yeasts in teat cups of milking machines was 20.5% and 51 strains were isolated (Table 1).

The 63 yeasts isolated from the milk and teat cup samples were classified into 58 strains of 9 species and unidentified 5 strains. *Candida* species were 84.1% of the isolates as in Table 2. From the milk samples, 11 strains of 4 *Candida* species and 1 *Rhodotorula* spp were isolated, and thus 91.7% of the isolates belonged to the genus *Candida*. *Candida* spe-

**Table 1.** Incidence of yeasts in milk of dairy cows with subclinical mastitis and teat cups in Chinju area

Item	No of sampled	No of positive (%)	No of isolates
Cows	161	12 ( 7.5)	
Quarters	330	12 ( 3.6)	12
Teat cups	200	41 (20.5)	51

**Table 2.** Species distribution of yeasts isolated from subclinical mastitis milk and teat cups

Species	No of isolates (%)		
	Milk	Teat cups	Total
<i>Candida pseudotropicalis</i>	4 (33.3)	13 (25.5)	17 (27.0)
<i>Candida tropicalis</i>	3 (25.0)	7 (13.7)	10 (15.9)
<i>Candida guilliermondii</i>		9 (17.6)	9 (14.3)
<i>Candida krusei</i>	2 (16.7)	5 ( 9.8)	7 (11.1)
<i>Candida albicans</i>	2 (16.7)	3 ( 5.9)	5 ( 7.9)
<i>Candida parapsilosis</i>		5 ( 9.8)	5 ( 7.9)
<i>Torulopsis glabrata</i>		2 ( 3.9)	2 ( 3.2)
<i>Geotrichum candidum</i>		2 ( 3.9)	2 ( 3.2)
<i>Rhodotorula</i> spp	1 ( 8.3)		1 ( 1.6)
Unidentified		5 ( 9.8)	5 ( 7.9)
Total	12 (100.0)	51 (100.0)	63 (100.0)

cies were found in order of prevalence of *C pseudotropicalis* (33.3%), *C tropicalis* (25.0%), *C albicans* (16.7%) and *C krusei* (16.7%). From the teat cups, 42 strains of 6 *Candida* species, each 2 of *Torulopsis glabrata* and *Geotrichum candidum* and 5 unidentified yeasts were isolated. *Candida* species were recovered in order of prevalence of *C pseudotropicalis* (25.5%), *C guilliermondii* (17.6%), *C tropicalis* (13.7%), *C krusei* (9.8%), *C parapsilosis* (9.8%) and *C albicans* (5.9%).

## Discussion

In the present study rate of subclinical yeast infection was 3.6% of 330 quarters in Chinju area (Table 1). This was lower than 18 (10.8%) of 166 quarters reported in the same area by Choi et al (1982)<sup>9</sup> but higher than 1.5% of 1,500 quarters in Taegu area.<sup>4</sup> These dissimilar findings were assumed due to the numerical differences in tested quarters,

and the infection rate shows a tendency to decrease in proportion to the diminution of test quarters.

*Candida* species were the predominant yeast as in 91.7% of 12 isolates from milk and made up 84.1% of 63 strains from both milk and teat cups (Table 2). Many earlier reports<sup>1,4,5,9,10,13</sup> revealed similar findings in milk or feces. Though the numbers of 4 *Candida* species isolated from the milk samples were 2 to 4 strains, *C pseudotropicalis* was most frequently encountered(33.3%) in the present study. However, Richard et al<sup>1</sup>, Farnsworth and Sorensen<sup>5</sup>, and Yeo and Choi<sup>4</sup> reported *C tropicalis* or *C krusei* as most frequent yeast but 2.2% to 9.1% *C pseudotropicalis*. Previously Choi et al<sup>9</sup> also isolated 9.5% *C pseudotropicalis* in Chinju area. The predominant species, therefore, seems to be influenced by the regional or seasonal variations. *C tropicalis* and *Rhodotorula* spp were also found in the milk, which were isolated in the region of Taegu<sup>4</sup> or Kangweon province<sup>10</sup> but were not isolated in Chinju area<sup>9</sup> previously.

In 20.5% of tested teat cups yeasts were contaminated(Table 1). The identified species were *C pseudotropicalis*, *C tropicalis*, *C krusei*, *C albicans* as well as those in the milk and *C guilliermondii*, *C parapsilosis*, *T glabrata* and *G candidum* which isolated solely from the teat cups(Table 2). It is apparent that these yeasts could be the infection source<sup>1</sup> and allow the development of mastitis if the other factors necessary for infection are present.

### Summary

The prevalence of yeasts in mammary glands of dairy cows and teat cups of milking machines was studied in Chinju area. The rate of subclinical yeast infection in 330 quarters was 3.6%.

Of 12 isolates from the milk, 4 *Candida pseudotropicalis*, 3 *C tropicalis*, 2 *C krusei*, 2 *C albicans* and 1 *Rhodotorula* spp were identified. The 91.7% of the isolates belonged to the genus *Candida* and *C pseudotropicalis* was the predominant species. From 20.5% of 200 teat cups tested, 51 strains of yeasts were isolated. These were 13 *C pseudotropicalis*, 9 *C guilliermondii*, 7 *C tropicalis*, 5 *C krusei*, 5 *C parapsilosis*, 3 *C albicans*, 2 *Torulopsis glabrata*, 2

*Geotrichum candidum* and 5 unidentified yeasts. *C pseudotropicalis* was most frequently encountered.

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