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Ecological Studies on the Causative Agents of Food Poisoning from Food Animals
1. Patterns on the Outbreaks and Antimicrobial Susceptibility of Causative Agents Isolated from Bovine Mastitis in a Rural Area

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畜產由來 食中毒 細菌에 關한 生態學的 研究
第 1 報：一部 農村地域 젖소 乳房炎의 發生樣相 및 그 原因菌이 抗菌療法劑에 關한 感受性

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국 문 초 록

전남지역에서 사육하고 있는 젖소 767두 중 유방염으로 의심되는 405두에서 원인균을 분리하여 유병률, 계절별 분리균의 분포, 항균 요법제에 대한 감수성 검사 등을 실시하였다. 유방염의 유병률을 살펴보면, 젖소별로는 총 767두 중에서 259두(33.8%)이었고, 분방별로는 총 3,068분방 중에서 568분방(18.5%)이었으며 계절별로는 8월(17.6%), 9월(12.7%), 4월(11.2%) 등의 순으로 나타났고 체세포 수자별로는 평균 $105.9\times10^4\pm79.5\times10^4$이었다. 원인균의 월별 분리빈도분포를 살펴보면, 1월에는 *Staphylococcus* sp. & *Escherichia coli*(33.3%), 2월 *Staphylococcus* sp.(71.4%), 3월 *Streptococcus* sp.(31.6%), 4월 *Streptococcus* sp.(41.4%), 5월 *Escherichia coli*(35.7%), 6월 *Staphylococcus aureus*(26.9%), 7월 *Salmonella* sp.(25.9%), 8월 *Escherichia coli*(20.5%), 9월 *Escherichia coli* & *Pseudomonas* sp.(21.2%), 10월 *Klebsiella* sp.(29.6%), 11월 *Salmonella* sp.(31.6%), 12월 *Streptococcus* sp.(37.5%) 등이 가장 높았다. 체세포 숫자별 원인균의 분리빈도 분포를 살펴보면, *Staphylococcus* sp.(251×10^4～300×10^4; 35.7%)을 제외하고는 일반적으로 50×10^4～100×10^4이 가장 높았다. 항균요법제에 대한 감수성은 일반적으로 trimethoprim/sulfamethoxazole은 *Streptococcus* sp., *Staphylococcus aureus*, *Staphylococcus* sp., *Klebsiella* sp.에 높았으며 gentamycin은 *Staphylococcus* sp., *Escherichia coli*, *Klebsiella* sp., *Proteus* sp., *Pseudomonas* sp., *Salmonella* sp.에 높았다.

Keywords : Outbreaks and Antimicrobial Susceptibility, Bovine Mastitis.

Introduction

Bovine mastitis is an economically and a hygienically important disease of dairy cows.[1,7] Many factors predispose to bovine mastitis and an understanding of these is essential for systems of effective mastitis control to be formulated.[2]

The presence of non-pathogenic bacteria on body surfaces can protect against invasion by more pathogenic organisms.[2] Bacteria of low pathogenicity (minor pathogens) are frequently isolated from the healthy bovine udder and may play an important role in protecting the udder from infection with pathogenic bacteria.[2]

The treatment of bovine mastitis is important for choosing adequate antimicrobias, and it takes the base on the result of susceptibility to antimicrobias.[3-5] Therefore, the current of numbers of feeding dairy cattle were increasing and prevalence rate of bovine mastitis was occurred in 1.5

Table 1. Outbreak patterns of bovine mastitis

Number of feeding (Quarter)	Number of request examined	Number of positive	
		Number of cow	Number or quarter
767 (3,068)	405 (52.8)*	259 (33.8)**	568 (18.5)**

*Percentage was calculated on the basis of number of feeding bovine.

$$**\text{Prevalence rate} = \frac{\text{Total number of cases, new or old, existing at that point in time}}{\text{Total population at that point in time}} \times 100$$

~57.3%.[6-8] In this study, species of causative agents on the bovine mastitis differentiated by an array of morphological, physiological, biochemical characters and antimicrobial susceptibility.

The present study aims at determining the adequate antimicrobias to bovine mastitis.

And also, this study looks forward to increasing for productivity of dairy farmer and promoting to nation's health through preventing food poisoning due to causative agents of bovine mastitis.

Materials and Methods

1. Test materials

A total of 259 samples of causative agents used in this study were isolated from raw milk of bovine mastitis among 405 samples to request for examining to Chonnam Animal Hygienic Center during the period from 4 January to 28 December in 1992 (Table 1).

2. Identification of causative agents

The positive was determined over 500,000 somatic cells per milliliter by Rolling Ball Viscometer (Made in New Zealand, RAI). Causative agents were identified to genus by colony morphology, characteristics on aesculin blood agar, gram stain, and biochemical examination (Fig. 1).

3. Susceptibility test

Antimicrobial susceptibility tests were performed following the modified Bauer-Kirby procedure recommended by National Committee Standard of Clinical Laboratory, U.S.A.,[9] and standardized disc susceptibility test by F.D.A. Regulation[10,11] (Table 6). Antimicrobias were chosen basing on activity against causative agents of bovine mastitis and included ampicillin, amikacin, bacitracin, carbenicillin, cephalothin, erythromycin, gentamycin, kanamycin, lincomycin, neomycin, nalidixin, penicillin, streptomycin, trimethoprim/sulfamethoxazole, and tetracycline (BBL and DIFCO Co., U.S.A.,

Table 2. Monthly distribution of the somatic cell in raw milk of bovine mastitis

Month \ Somatic cell (×10⁴/ml)	Number of positive (%)*	Mean (X)	Standard deviation (S.D.)	Coefficient of variation (C.V.)	Range
Jan.	6(2.3)	130.2	62.6	48.1	50~300
Feb.	7(2.7)	135.7	88.8	65.4	50~300
Mar.	19(7.3)	244.5	109.9	44.9	50~350
Apr.	29(11.2)	227.9	101.3	44.4	50~300
May	14(5.4)	176.1	117.2	66.6	50~300
Jun.	26(10.0)	194.4	105.5	54.3	50~300
Jul.	27(10.4)	135.2	85.3	63.1	50~300
Aug.	44(17.0)	178.6	91.3	51.1	50~300
Sep.	33(12.7)	120.8	86.3	71.4	50~350
Oct.	27(10.4)	114.1	51.9	54.5	50~200
Nov.	19(7.3)	88.4	37.6	42.5	50~200
Dec.	8(3.1)	65.0	16.0	24.6	50~ 80
Total	259(100.0)	150.9	79.5	52.6	

*Number of positive : over 500,000 cells per milliliter determined by rolling ball viscosity test (R.B.V.T.).

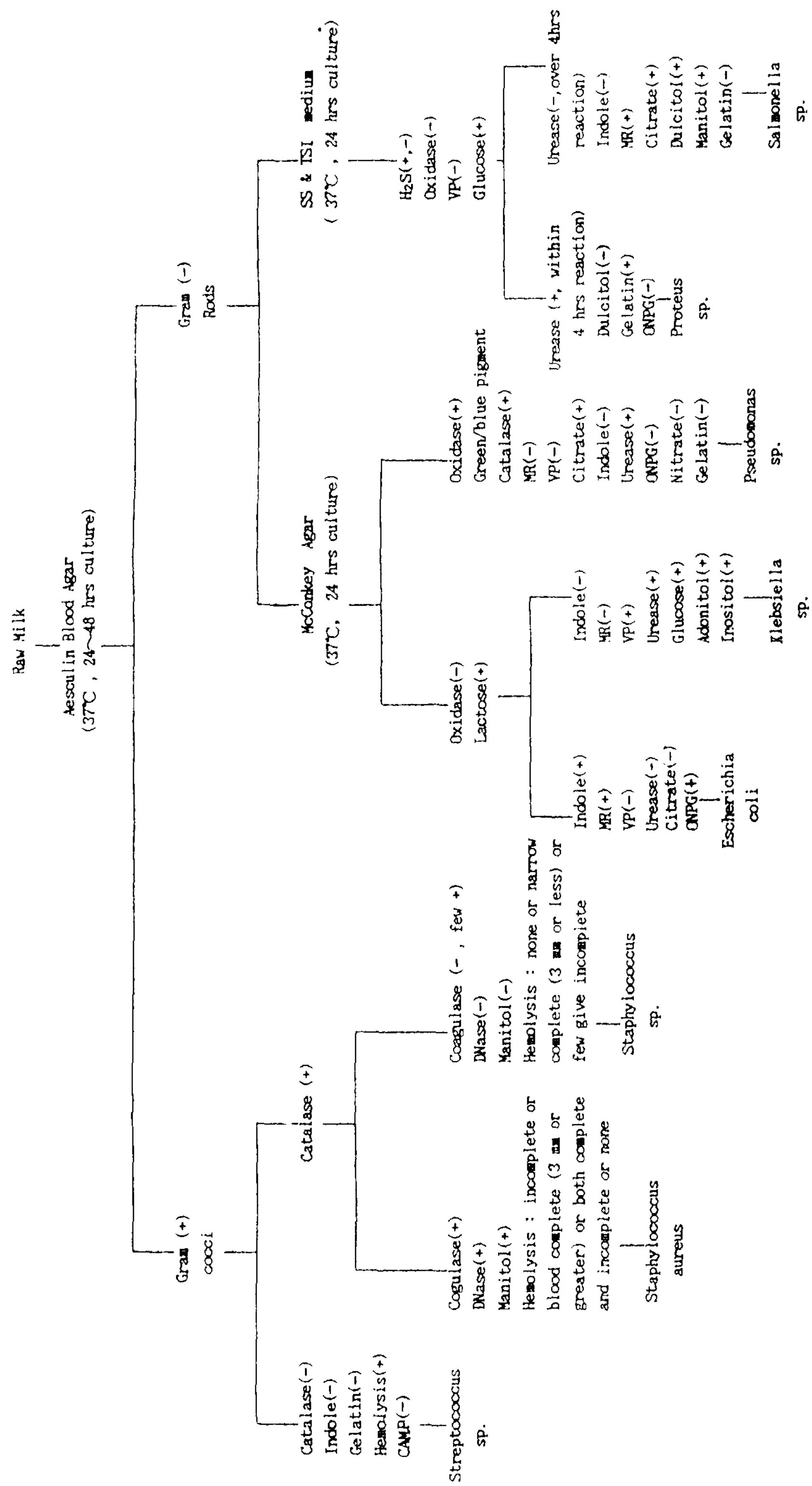

Fig. 1. Methods of identification of causative agents isolated from raw milk of bovine mastitis.

Table 3. Monthly distribution of the somatic cell number in raw milk of bovine mastitis (%)

Somatic cell (×10⁴/ml) / Month	50～100	101～150	151～200	201～250	251～300	301～	Total
Jan.	3(50.0)	2(33.3)	–	–	1(16.7)	–	6(100.0)
Feb.	3(42.9)	2(28.6)	1(14.3)	–	1(14.3)	–	7(100.0)
Mar.	3(15.8)	3(15.8)	1(5.3)	–	7(36.8)	5(26.3)	19(100.0)
Apr.	7(24.1)	1(3.4)	3(10.3)	–	18(62.1)	–	29(100.0)
May	7(50.0)	–	1(7.1)	–	6(42.9)	–	14(100.0)
Jun.	8(30.8)	5(19.2)	1(3.8)	–	12(46.2)	–	26(100.0)
Jul.	16(59.3)	5(18.5)	1(3.7)	1(3.7)	4(14.8)	–	27(100.0)
Aug.	15(34.1)	12(27.3)	3(6.8)	–	14(31.8)	–	44(100.0)
Sep.	22(66.7)	5(15.2)	1(3.0)	1(3.0)	3(9.0)	1(3.0)	33(100.0)
Oct.	17(63.0)	5(18.5)	5(18.5)	–	–	–	27(100.0)
Nov.	17(89.5)	1(5.3)	1(5.3)	–	–	–	19(100.0)
Dec.	8(100.0)	–	–	–	–	–	8(100.0)
Total	126(48.6)	41(15.8)	18(6.9)	2(0.8)	66(25.5)	6(2.3)	259(100.0)

Table 4. Monthly distribution of the causative agents isolated from raw milk of bovine mastitis (%)

Causative agent / Month	*Streptococcus* sp.	*Staphylococcus aureus*	*Staphylococcus* sp.	*Escherichia coli*	*Klebsiella* sp.	*Proteus* sp.	*Pseudomonas* sp.	*Salmonella* sp.	Total
Jan.	1(16.7)	1(16.7)	2(33.3)	2(33.3)	–	–	–	–	6(100.0)
Feb.	1(14.3)	–	5(71.4)	1(14.3)	–	–	–	–	7(100.0)
Mar.	6(31.6)	3(15.8)	3(15.8)	4(21.1)	2(10.5)	–	–	1(5.3)	19(100.0)
Apr.	12(41.4)	6(20.7)	1(3.4)	3(10.3)	2(6.9)	–	1(3.4)	4(13.8)	29(100.0)
May	2(14.3)	1(7.1)	1(7.1)	5(35.7)	2(14.3)	1(7.1)	1(7.1)	1(7.1)	14(100.0)
Jun.	4(15.4)	7(26.9)	4(15.4)	3(11.5)	1(3.8)	–	2(7.7)	5(19.2)	26(100.0)
Jul.	6(22.2)	4(14.8)	2(7.4)	2(7.4)	1(3.7)	3(11.1)	2(7.4)	7(25.9)	27(100.0)
Aug.	8(18.2)	4(9.1)	6(13.6)	9(20.5)	5(11.4)	3(6.8)	7(15.9)	2(4.5)	44(100.0)
Sep.	4(12.1)	2(6.1)	2(6.1)	7(21.2)	4(12.1)	5(15.2)	7(21.2)	2(6.1)	33(100.0)
Oct.	2(7.4)	5(18.5)	1(3.7)	4(14.8)	8(29.6)	4(14.8)	3(11.1)	–	27(100.0)
Nov.	3(15.8)	2(10.5)	1(5.3)	1(5.3)	2(10.5)	–	4(21.1)	6(31.6)	19(100.0)
Dec.	3(37.5)	–	–	2(25.0)	–	2(25.0)	–	1(12.5)	8(100.0)
Total	52(20.1)	35(13.5)	28(10.8)	43(16.6)	27(10.4)	18(6.9)	27(10.4)	29(11.2)	259(100.0)

Table 6).

Results

The prevalence rate of the bovine mastitis from raw milk examined was observed in 259 samples (33.8%) among the feeding dairy cows of 767 on dairy farms and quarters of 568 (18.5%) among quarters of 3,068 by raw milk examining (Table 1).

The occurrence frequencies by seasons of bovine mastitis were observed in the order of August (17.0%), September (12.7%), April (11.2%), July (10.4%), and October (10.4%) etc. (Table 2).

The mean of positive according to somatic cell number was observed in the order of March ($244.5\times10^4\pm109.9\times10^4$), April ($227.9\times10^4\pm101.3\times10^4$), June ($194.4\times10^4\pm105.5\times10^4$), August ($178.6\times10^4\pm91.3\times10^4$) etc., and overall mean was observed in $105.9\times10^4\pm79.5\times10^4$ (Table 2).

The monthly distribution according to somatic cell number was observed in the highest of 50～100×10^4 generally except March (251～300×10^4;

Table 5. Distribution of the causative agents isolated from raw milk of bovine mastitis by somatic cell number (%)

Causative agent / Somatic cell (×10⁴/ml)	*Streptococcus* sp.	*Staphylococcus aureus*	*Staphylococcus* sp.	*Escherichia coli*	*Klebsiella* sp.	*Proteus* sp.	*Pseudomonas* sp.	*Salmonella* sp.	Total
50~100	21(40.4)	16(45.6)	9(32.1)	21(48.8)	16(59.3)	13(72.2)	15(55.6)	15(51.7)	126(48.6)
101~150	7(13.5)	5(14.3)	5(17.9)	8(18.6)	2(7.4)	2(11.1)	7(25.9)	5(17.2)	41(15.8)
151~200	4(7.7)	3(8.6)	3(10.7)	2(4.7)	2(7.4)	–	2(7.4)	2(6.9)	18(6.9)
201~250	1(1.9)	–	–	–	–	–	–	1(3.4)	2(0.8)
251~300	17(32.7)	9(25.7)	10(35.7)	12(27.9)	7(25.9)	3(16.7)	3(11.1)	5(17.2)	66(25.5)
301~	2(3.8)	2(5.7)	1(3.6)	–	–	–	–	1(3.4)	6(2.3)
Total	52(100.0)	35(100.0)	28(100.0)	43(100.0)	27(100.0)	18(100.0)	27(100.0)	29(100.0)	259(100.0)

Table 6. Antimicrobial susceptibility of the causative agents isolated from raw milk of bovine mastitis (%)

Antimicrobia	*Disc potency*	Diameter of susceptible zone	*Streptococcus* sp.	*Staphylococcus aureus*	*Staphylococcus* sp.	*Escherichia coli*	*Klebsiella* sp.	*Proteus* sp.	*Pseudomonas* sp.	*Salmonella* sp.
Total (No. of sample)	mcg or unit	mm or more	52(100.0)	35(100.0)	28(100.0)	43(100.0)	27(100.0)	18(100.0)	27(100.0)	29(100.0)
Ampicillin(AM)	10 mcg	17	22(42.3)	8(22.9)	6(21.4)	5(11.6)	1(3.7)	1(5.6)	1(3.7)	–
Amikacin(AN)	30 mcg	14	6(11.5)	2(5.7)	6(21.4)	10(23.3)	3(11.1)	2(11.1)	6(22.2)	4(13.8)
Bacitracin(BA)	10 unit	13	1(1.9)	–	1(3.6)	2(4.7)	–	–	–	–
Carbenicillin(CB)	100 mcg	20~24 (*Proteus* sp. & *E. coli* 23, pseudomonas 17)	4(7.7)	4(11.4)	–	–	–	1(5.6)	2(7.4)	2(6.9)
Cephalothin(CF)	30 mcg	18	9(17.3)	12(34.3)	9(32.1)	4(9.3)	6(22.2)	2(11.1)	3(11.1)	1(3.4)
Erythromycin (EM)	15 mcg	18	10(19.2)	11(31.4)	4(14.3)	3(7.0)	2(7.4)	2(11.1)	–	–
Gentamycin(GM)	10 mcg	13	11(21.2)	20(57.1)	12(42.9)	21(48.9)	14(51.9)	10(55.6)	18(66.7)	17(58.6)
Kanamycin(KM)	30 mcg	18	12(23.1)	9(25.7)	4(14.3)	7(16.3)	12(44.4)	7(38.9)	2(7.4)	6(20.7)
Lincomycin(LM)	2 mcg	17	1(1.9)	–	1(3.6)	–	–	–	–	–
Neomycin(NM)	30 mcg	17	5(9.6)	11(31.4)	6(21.4)	7(16.3)	10(37.0)	3(16.7)	8(29.6)	8(27.6)
Nalidixin(NA)	30 mcg	19	1(1.9)	[illegible]	[illegible]	[illegible]				–
Penicillin(PP)	10 unit	22	4(7.7)	3(8.6)	1(3.6)	–	2(7.4)	1(5.6)	–	1(3.4)
Streptomycin (SM)	10 mcg	15	3(5.8)	4(11.4)	–	1(2.3)	1(3.7)	2(11.1)	6(22.2)	3(10.3)
Trimethoprim/ sulfamethoxazole (SXT)	1.25± 23.75 mcg	16	28(53.8)	26(74.3)	12(42.9)	16(37.2)	14(51.9)	7(38.9)	2(7.4)	15(51.7)
Tetracycline(TC)	30 mcg	19	6(11.5)	10(28.6)	2(7.1)	8(18.6)	1(3.7)	7(38.9)	–	3(10.3)

36.8%), April ($251\sim300\times10^4$; 62.1%), and June ($251\sim300\times10^4$; 46.2%) (Table 3).

The monthly distribution of causative agents was observed in the highest of both *Staphylococcus* sp. (33.3%) and *Escherichia coli* (33.3%) on January, *Staphylococcus* sp. (71.4%) on February, *Streptococcus* sp. (31.6%) on March, *Streptococcus* sp. (41.4%) on April, *Escherichia coli* (35.7%) on May, *Staphylococcus aureus* (26.9%) on June, *Salmonella* sp. (25.9%) on July, *Escherichia coli* (20.5%) on August, both *Escherichia coli* (21.2%) and *Pseudomonas* sp. (21.2%) on September, *Klebsiella* sp. (29.6 %) on October, *Salmonella* sp. (31.6%) on November, and *Streptococcus* sp. (37.5%) on December respectively (Table 4).

The distribution of causative agents according to somatic cell number was observed in the highest of $50\sim100\times10^4$ generally, only except *Staphylococcus* sp. ($251\sim300\times10^4$; 35.7%) (Table 5).

The susceptibility of antimicrobias to the causative agents was observed in the order of trimethoprim/sulfamethoxazole (SXT; 53.8%), ampicillin (AM; 42.3%), and kanamycin (KM; 23.1%) to *Streptococcus* sp., trimethoprim/sulfamethoxazole (SXT; 74.3%), gentamycin (GM; 57.1%) and cephalothin (CF; 34.3%) to *Staphylococcus aureus*, both gentamycin (GM; 42.9%), and trimethoprim/sulfamethoxazole (SXT; 42.9%), and cephalothin (CF; 32.1 %) to *Staphylococcus* sp., gentamycin (GM; 48.9%), trimethoprim/sulfamethoxazole (SXT; 37.2%), and amikacin (AN; 23.3%) to *Escherichia coli*, both gentamycin (GM; 51.9%), and trimethoprim/sulfamethoxazole (SXT; 51.9%) and kanamycin (KM; 44.4%) to *Klebsiella* sp., gentamycin (GM; 55.6%), both kanamycin (KM; 38.9%) and trimethoprim/sulfamethoxazole (SXT; 38.9%) to *Proteus* sp., gentamycin (GM; 66.7%), neomycin (NM; 29.6%), both amikacin (AN; 22.2%), and streptomycin (SM; 22.2%) to *Pseudomonas* sp., gentamycin (GM; 58.6%), trimethoprim/sulfamethoxazole (SXT; 51.7 %) and neomycin (NM; 27.6%) to *Salmonella* sp., respectively (Table 6).

Discussion

In this study, the prevalence rate of bovine mastitis from raw milk examined was observed in 259 samples (33.8%) among the feeding cows of 767 on dairy farms and quarters of 568 (18.5%) among quarters of 3,068 by raw milk examining.

The occurrence frequencies by seasons of bovine mastitis were observed in the order of August (17.0%), September (12.7%), April (11.2%), July (10.4%), and October (10.4%).

Jung (1987) reported that incidence rate of bovine mastitis from raw milk examined was observed in dairy cows of 1,393 (18.8%) among the cows of 7,406 on dairy farms (7).

These outbreaks by years were increased to 17.1% in 1984, 19.7% in 1985, and 20.5% in 1986. And outbreaks of bovine mastitis by seasons were highly observed during the period from August to October in 1987 by Jung.[7]

This suggests that the bovine mastitis shows a trend to increase annually.

Na and Kang (1975) reported that the clinical mastitis was found at 7 quarters (3.5%) of 5 cows (5.0%).[12]

Of the 739 samples, 24.4% had positive D. M. L. C. (direct microscopic leucocyte count) value (over 500,000 leucocytes per milliliter), 32.6% positive M. C. M. T. (modified california mastitis test) reaction, 34.9% positive R. R. T. (resazurin reduction test) reaction and 39.9% positive M. W. T. (modified whiteside test) reaction in 1975 by Na.[13]

Average total bacterial count of the 119 raw milk samples was 131,900 cells per milliliter and the incidence rate of abnormal milk was observed in 31.9% in 1974 by Kim and Kang.[14]

Han (1986) reported that one hundred and fifty three (57.3%) of 267 cows were clinical mastitis and 204 (20.4%) of 998 quarters were in subclinical mastitis.[6]

Observations with 910 cows on dairy farms showed 1.5% clinical form and 41.6% subclinical form in the infections rate of bovine mastitis according to the degree of infection in 1989 by Kim.[8]

As the result, we could find it was apparent difference in region and year.

In this study, the mean of positive according to somatic cell number was observed in the order of March ($244.5\times10^4\pm109.9\times10^4$), April ($227.9\times10^4\pm/101.3\times10^4$), June ($194.4\times10^4\pm105.5\times10^4$), August ($178.6\times10^4\pm91.3\times10^4$), overall mean was observed $105.9\times10^4\pm79.5\times10^4$.

The monthly distribution according to somatic cell number was observed in the highest of $50\times10^4\sim100\times10^4$ generally except March ($251\times10^4\sim300\times10^4$; 36.8%), April ($251\times10^4\sim300\times10^4$; 62.1%), and June ($251\times10^4\sim300\times10^4$; 46.2%).

The distribution of causative agents according to somatic cell number was observed in the highest of $50\times10^4\sim100\times10^4$ in general, only except *Staphylococcus* sp. ($251\times10^4\sim300\times10^4$; 35.7%).

Lee *et al.* (1987) reported that the mean of positive according to the mean of somatic cell number was observed in $2{,}093.3\pm185.4\times10^3$ per milliliter.[15)]

On the other hand of the 565 cows examined, infectious ratio of subclinical mastitis by both screening tests was 43.9% in R.B.V.T. and 40.0% in C.M.T. and the ratio R.B.V.T. and C.M.T. positive reaction among 2,204 quarters was 19.0% and 17.1% respectively in 1987 by Park *et al.*[16)]

In this study, the monthly distribution of causative agents was observed in the highest of both *Staphylococcus* sp. (33.3%) and *Escherichia coli* (33.3%) on January, *Staphylococcus* sp. (71.4%) on February, *Streptococcus* sp. (31.6%) on march, *Streptococcus* sp. (41.4%) on April, *Escherichia coli* (35.7%) on May, *Staphylococcus aureus* (26.9%) on June, *Salmonella* sp. (25.9%) on July, *Escherichia coli* (20.5%) on August, both *Escherichia coli* (21.2%) and *Pseudomonas* sp. (21.2%) on September, *Klebsiella* sp. (29.6%) on October, *Salmonella* sp. (31.6%) on November, and *Streptococcus* sp. (37.5%) on December respectively.

Jung (1987) reported that isolated rate of mastitis causative agents from raw milk was observed in the order of *Staphylococcus* sp. (50.2~51.7%), *Streptococcus* sp. (35.6~48.3%), *Bacillus* sp. (2.2~6.7%), *Pseudomonas* sp. (0.3~5.7%), and others (0.6%).[7)]

It's known that bovine mastitis is caused by infection with various bacteria and mycoplasmas. In recent years, cases of bacterial mastitis caused by Streptococci are gradually decreasing and bovine mastitis by *Staphylococcus aureus* are increasing in 1983 by Takeshige *et al.*[17)]

Han (1986) reported that among 431 quarters, 404 quarters were infected with *Staphylococcus aureus* (34.7%), non-*Streptococcus agalactiae* (14.9%), *Staphylococcus epidermidis* (12.8%), coliform organisms (10.7%) and yeast (10.2%), one or supper infection and 27 quarters were not isolated.[6)]

On the other hand, isolation of major known mammary pathogens compared to *Staphylococcus* sp. (40.7%), non-identified gram negative rods (33.2%) and *E. coli* (7.4%).

In the mixed infection, *Staphylococcus* sp. was most frequently involved in 1980 by Lee.[18)]

As the result, it was apparent difference by region, year and symptom of case.

In this study, the susceptibility of antimicrobials to the causative agents was observed in the highest of trimethoprim/sulfamethoxazole (SXT) to *Streptococcus* sp., *Staphylococcus aureus*, *Staphylococcus* sp., and *Klebsiella* sp., but gentamycin to *Staphylococcus* sp., *Escherichia coli*, *Klebsiella* sp., *Proteus* sp., *Pseudomonas* sp., and *Salmonella* sp. in general.

Jung (1987) reported that antibiotics susceptibility of isolated bovine mastitis agents from raw milk was observed high susceptible in cephalosporin, novobiocin, penicillin, streptomycin, neomycin, erythromycin, gentamycin.[7)]

Kim (1974) reported that the number of resistant strain among 60 strains of *Staphylococcus aureus* to penicillin (35.0%), Streptomycin (8.3%), neomycin (80.0%), novobiocin (11.7%), oxytetracyclin (23.3%), and erythromycin (76.7%) respectively.[19)]

Kim (1984) reported that the combination of cloxacillin with gentamycin showed a synergistic effect by inhibiting regrowth of the tested organism.[20)]

And Ha (1990) reported that all strains of *Escherichia coli* were susceptible to amikacin and moxalactam 1.9~3.9% of the strains were resistant to cefamandole, nalidixic acid, and rifampin, and 18.3~21.2% were resistant to gentamycin, and cephalothin 41.3~57.7% were resistant to kanamycin, streptomycin, and trimethoprim 67.6~83.7% were resistant to penicillin, ampicillin, tetracycline and sulfamethoxazole.[21]

And then, Shin *et al.* (1990) reported that *Pseudomonas* sp., *Citrobacter* sp. and *Serratia* sp., were high resistant to β-lactams but no effects by enzyme inhibitors were observed.[22]

On the other hand, Han (1990) reported that the susceptibilities of organism to 11 drugs examined were found in the highest susceptibilities to kanamycin, ampicillin, and bacampicillin but lowest to tetracycline, erythromycin, and sulfamethoxazole in order.[23]

As the result, it was apparent difference by strain and kind of antimicrobia.

Summary

Patterns on the outbreaks and antimicrobial susceptibility by causative agents isolated from raw milk of bovine mastitis were identified on 259 samples among the 405 by the somatic cells, colony morphology, characteristics on aesculin blood agar, gram stain and biochemical examination in Chonnam, Korea during the period from 4 January to 28 December in 1992.

The results are summarized as follows :

1. Prevalence rate of the bovine mastitis was observed in dairy cows of 259 (33.8%) among dairy cows of 767 on dairy farms and quarters of 568 (18.5%) among quarters of 3,068 by raw milk examining.
   These occurrence frequencies by season of bovine mastitis were observed in the order of August (17.0%), September (12.7%), April (11.2%), July (10.4%), and October (10.4%).
2. The mean of positive according to somatic cell number was observed in the order of March ($244.5\times10^4\pm109.9\times10^4$), April ($227.9\times10^4\pm101.3\pm10^4$), June ($194.4\times10^4\pm105.5\times10^4$), August ($178.6\times10^4\pm91.3\times10^4$), and overall mean was observed in $105.9\times10^4\pm79.5\times10^4$.
3. The monthly distribution of causative agents was observed in the highest of both *Staphylococcus* sp. (33.3%) and *Escherichia coli* (33.3%) in January. *Staphylococcs* sp. (71.4%) in February, *Streptococcus* sp. (31.6%) in March, *Streptococcus* sp. (41.4%) in April. *Escherichia coli* (35.7%) in May, *Staphylococcus aureus* (26.9%) in June, *Salmonella* sp. (25.9%) in July, *Escherichia coli* (20.5%) in August, both *Escherichia coli* (21.2%) and *Pseudomonas* sp. (21.2%) in September, *Klebsiella* sp. (29.6%) in October, *Salmonella* sp. (31.6%) in November, and *Streptococcus* sp. (37.5%) in December respectively.
4. The distribution of causative agents according to somatic cell number was observed in the highest of $50\times10^4\sim100\times10^4$ in general, only except *Staphylococcus* sp. ($251\times10^4\sim300\times10^4$; 35.7%).
5. The susceptibility to antimicrobias of the causative agents was observed in the highest of trimethoprim/sulfamethoxazole to *Streptococcus* sp., *Staphylococcus aureus, Staphylococcus* sp., and *Klebsiella* sp. but gentamycin to *Staphylococcus* sp., *Escherichia coli, Klebsiella* sp., *Proteus* sp., *Pseudomonas* sp., *Salmonella sp. in general.*

References

1) Hazlett, M. J., Little, P. B., Maxie, M. G. and Barnum, D. A. : Fatal Mastitis of Dairy Cows: A Retrospective Study. *Can J. Comp. Med.*, **48**, 125-129, 1984.

2) Robinson, T. C., Jackon, E. R. and Marr, A. : Mastitis Incidence in Quarters with Different Infection Status at Drying off and Calving in Two Treatment Goups. *Br. Vet. J.*, **144**, 166-173, 1988.

3) Craven, N. : Efficacy and Financial Value of Antibiotic Treatment of Bovine Clinical Mastitis During Lactation-*A Review. Br. Vet. J.*, **143**, 410-422, 1987.

4) Hinckley, L. S., Benson, R. H., Post, J. E. and DeCloux, J. C. : Antibiotic Susceptibility Profiles for Mastitis Treatment. *J. Am. Vet. Med. Assoc.*, **187**, 700-711, 1985.

5) Kim, D. : Patterns of Mastitic Pathogens and Antibiotic Susceptibility of Bovine Clinical Mastitis. *Korean J. Vet. Res.*, **28**(2), 397-404, 1988.

6) Han, K. S. : The Bovine Mastitis Status in Chonbuk Province. *J. Korean Vet. Med. Assoc.*, **22**(10), 633-637, 1986.

7) Jung, H. K. : Epidemiological Study on the Bovine Mastitis in Chonnam Area. *Korean J. Env. Hlth. Soc.*, **13**(2), 75-81, 1987.
8) Kim, T. J. : Studies on the Isolation of Streptococcal Species Causing Bovine Mastitis and Susceptibility Test to Them. *J. Korean Vet. Med. Assoc.*, **25**(7), 423-433, 1989.
9) Owens, W. E. and Watts, J. L. : Antimicrobial Susceptibility and β-Lactamase Testing of Staphylococci Isolated from Dairy Herds. *J. Dairy Sci.*, **71**, 1934-1939, 1988.
10) F. D. A. : Standardized Disc Susceptibility Test. F. D. A. Regulation, April, 1, 680, 1976.
11) Steers, E., Flotz, E. L. and Graves, B. S. : An Inocular Replicating Apparatus for Routine Testing of Bacterial Susceptibility to Antibiotics. *Antibiot. Chemother.*, **9**, 307-311, 1959.
12) Na, J. S. and Kang, B. K. : Studies on Epidemiological Investigations of Bovine Mastitis in Jeonnam District. I. Total Bacterial Count of Raw Milk and Survey of Bovine Mastitis. *Korean J. Vet. Res.*, **15**(1), 83-91, 1975.
13) Na, J. S. : Studies on Epidemiological Investigations of Bovine Mastitis in Jeonnam District. *Korean J. Vet. Res.*, **15**(1), 93-99, 1975.
14) Kim, H. K. and Kang, B. K. : Studies on Epidemiological Investigation of Mastitis in the Cows of Chonnam Area. *The Research Reports of Chonnam Provincial Office of Rural Development*, **2**, 57-65, 1974.
15) Lee, J. S., Kang, B. K., Park, Y. J. and Lee, C. G. : Somatic Cell Counts and Blood Chemical Values of the Bovine Mastitis. *J. Korean Vet. Med. Assoc.*, **23**(10), 663-670, 1987.
16) Park, D. S., Ha, Y. J., Lee, J. H., Cho, H. T. and Kang, H. J. : Comparisons of the Rolling Ball Viscometer Test and California Mastitis Test for the Subclinical Mastitis Screening Test of Dairy Cow. *J. Korean Vet. Med. Assoc.*, **23**(8), 537-543, 1987.
17) Takeshige Kuniko, Watanabe Kenya, Igarashi Hideo, Shingaki Masao and Terayama Takashi : Detection of *Staphylococcus aureus* in Bovine Mastitis and Some Characteristics with Special Reference to Enterotoxin Producibility and Coagulase Types of Isolates. *Jpn. J. Vet. Sci.*, **45**(3), 355-362, 1983.
18) Lee, C. Y., Kang, B. K., Park, Y. J. and Lee, C. G. : Mastitis in Slaughtered Dairy Cows. I. Udder Infection. *Korean J. Vet. Res.*, **20**(2), 119-122, 1980.
19) Kim, Y. S. : A Study on the Sensitivity of *Staphylococcus aureus* and *Streptococcus* sp., Causative Organism of Bovine Mastitis Aginst Various Antibiotics. *Korean J. Pub. Hlth.*, **11**(2), 259-264, 1974.
20) Park, C. K. : Studies on Staphylococci Isolated from Bovine Mastitis. III. Activity of Penicillins and Gentamycin to Isolates. *Korean J. Vet. Res.*, **24**(2), 163-168, 1984.
21) Ha, K. I., Suh, S. I., Park, J. W. and Suh, M. H. : Characterization of R Plasmid and Antimicrobial Drug Resistance of *Escherichia coli*. *J. Korean Soc. Microbiol.*, **25**(1), 19-26, 1990.
22) Shin, K. *et al.* : Susceptibility Patterns to β-Lactam Antibiotics and the Effects of β-Lactamase Inhibition on Various Gram Negative Enteric Rods. *J. Korean Soc. Microbiol.*, **25**(4), 313-318, 1990.
23) Han, W. S. : Antimicrobial Susceptibility of *Escherichia coli* Isolated from Children and Chickens. *J. Korean Soc. Microbiol.*, **25**(5), 399-408, 1990.