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Patterns on the Outbreaks of Bovine Mastitis and Susceptibility to Antimicrobias of Isolated Causative Agents in a Municipal Area

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一部 都市地域 젖소 乳房炎의 發生樣相 및 그 原因菌의 抗菌療法 劑에 關한 感受性

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국문요약

광주시 지역에서 사육하고 있는 젖소 1,614두중 유방염으로 의심되는 730분방 중에서 170분방을 검사분석하여 유병률, 균의 분리와 간이검사법과의 관계, 계절별 분리균의 분포, 항균 요법제에 대한 감수성 등을 검사하였다. 본 원인균은 730분방 중 134분방(18.4%)에서 분리되었으며 체세포 숫자는 평균 $1.620\times10^6\pm1.167\times10^6$/ml(C.V.; 72.0%)이었다. CMT 반응치는 평균 2.9± 1.2(C.V.; 41.4%)이었으며 WT 반응치는 평균 2.8± 1.2(C.V.; 42.9%)이었다. RBVT와 CMT의 상관계수는 0.82($P<0.001$)이었고 RBVT와 WT의 상관계수는 0.75 ($P<0.001$)이었으며 CMT와 WT의 상관계수는 0.93 ($P<0.001$)이었다. 체세포 숫자를 기준으로 하여 CMT 및 WT의 양성율을 비교하여 보면 원인균이 분리된 경우에는 체세포 숫자가 0.49×10^6 이하/ml의 경우에 반응치가 1+일 때의 CMT는 72.4%, WT는 42.1%이었고 체세포 숫자가 $0.50\times10^6\sim1.00\times10^6$/ml의 경우에 반응치가 2+일 때의 CMT는 45.5%, WT는 48.8%이었으며, 체세포 숫자가 3.01×10^6 이상/ml의 경우에 반응치가 3+일 때의 CMT는 73.7%, WT는 92.3%이었다. 원인균의 월별 분리 빈도를 보면 8월 (17.9%)이 가장 높았고 다음은 9월(16.4%), 7월 (12.7%), 6월 (11.2%), 1월 (9.0%)의 순이었다. 원인균의 분리 빈도를 보면 *Staphylococcus* sp. (51.4%)가 가장 높았고 다음은 *Escherichia coli*(23.9%), *Pseudomonas* sp. (11.2%). *Streptococcus* sp. (6.7%)의 순이었다. 항균 요법제에 대한 감수성은 trimethoprim/sulfamethoxazole은 *Streptococcus* sp., *Staphylococcus epidermidis*, *Proteus* sp., *Salmonella* sp. 등에 높았고 gentamycin은 *Streptococcus* sp., *Staphylococcus aureus*, *Enterobacteriaceae*, *Klebsiella* sp., *Proteus* sp., *Salmonella* sp. 등에 높았으며 enrofloxacin은 일반적으로 거의 모든 균에서 감수성이 높았다.

Keywords: Outbreaks, Screening Test and Antimicrobial Susceptibility, Bovine Mastitis.

I. Introduction

The bovine mastitis is an economically and a hygienically important disease of dairy cows.[1,4,5] The bovine mastitis occurred differently in according to strain, causative agents, transmission, pathogenicity, season, annual, and area.[6] The control of mastitis in dairy herds has always consisted of surveillance, so that the mastitis status of the herd could be assessed. and the application of preventive techniques to reduce the quarter infection rate. A mastitis control program should not be looked upon as a rigidly enforceable set of rules. There are a number of basic techiques, and each of which can be enforced with varying degrees of strictness. And a number of additional techniques and each of which provide a small additional gain and which is usually applicable in a certain set of circumstances. The Food and Drug Administration (FDA) considers antibiotic-contaminated milk adulterated. Such adulteration can be minimized by exclusion of contaminated milk from the general milk supply. The FDA has attempted to reduce adulteration by limiting the quantity of antibiotic in each preparation to be used for mastitis therapy and by a requirement that a warning against the use of milk from recently treated animals be placed on the preparation.[2-4] The treatment of bovine mastitis is important for choosing adequate antimicrobias, and it takes the base on result of susceptibility to antimicrobias.[5,7-9] In this study, species of causative agents on the bovine mastitis were differentiated by an array of morphological, physiological, biochemical characters, antimicrobial suscep tibility, relation among rolling ball viscosity test, california mastitis thest and whiteside test. And also, this study looks forward to increasing for productivity of dairy farmer and promotion to nation's health through preventing food posioning due to causative agents of bovine mastitis.

Materials and Methods

Test materials

A total samples of 730 in this study were examined for isolation to causative agents from raw milk of quarters of bovine mastitis among quarters of 6, 456 in Kwang-ju area from 1992 to 1993 (Table 1).

Identification of causative agents

The screening tests were determined by rolling ball viscosity test(RBVT; Rolling Ball Viscometer, Made in New Zealand, RAI), california mastitis test (CMT), and witeside test (WT). Causative agents were identified to genus by colony morphology, characteristics on aesculin blood agar, gram stain, and biochemical examination.[5]

Susceptibility test

Antimicrobial susceptibility test was performed as standardized disc susceptibility test.[5,10-12] Antimicrobias were chosen basing on activity against causative agents of bovine mastitis and included ampicillin, cephalothin, chloxacillin, enrofloxacin, erythromycin, gentamycin, kanamycin, lincomycin, neomycin, penicillin, streptomycin, tetracycline, and trimethoprim/sulfamethoxazole (BBL and DIFCO Co., U.S.A).

Results

Patterns on the outbreaks of bovine mastitis by examining of raw milk was observed in subclinical cows of 323(20.0%), clinical cows of 70(4.3%) among the feeding dairy cows of 1,614 on dairy farms, and then subclinical quarters of 643(10.0%), clinical quarters of 87(1.3%) among the quarters of 6,456 in Table 1. The causative agents were isolated from raw milk of quarters of 134(18.4%) among the quarters of 730 (Table 1). The arithmetic mean of somatic cell number was observed in $1.62\times10^6\pm1.167\times10^6$ per milliliter (coefficient of variation: 72.0%) by rolling ball viscosity test (RBVT) in Table 2. The mean of reaction values was observed in 2.9± 1.2 (coefficient of variation; 41.4%) by california mastitis test(CMT), and 2.8± 1.2 (coefficient of variation: 42.9%) by whiteside test(WT) (Table 2). The correlation coefficient was distributed in 0.82($P<0.001$) by relation between rolling ball viscosity test (somatic cell$\times10^6$ per milliliter) and calfornia mastitis test, 0.75($P<0.001$) by relation between rolling ball viscosity test (somatic cell$\times10^6$ permilliliter) and whiteside test, and then 0.93($P<0.001$) by relation between california mastitis test and whiteside test in Table 3. However, significant comparison of excepting

Table 1. Patterns of outbreaks on the bovine mastitis(%)

Number of feeding bovine	Number of quarter	Number of infected cow		Number of infected quarter		Number of examined	
		subclinical	clinical	subclinical	clinical	Causative agent isolated	Causative agent non-isolated
1.614	6,456	323(20.0)	70(4.3)	643(10.0)	87(1.3)*	134(18.4)*	36(4.9)*·

* Percentage was calculated on the basis of infected quarters of 730 (subclinical; quarters of 643, clinical; quarters of 87).

Table 2. Distribution of reaction values by screening tests of raw milk of quarters in bovine mastitis(%)

Screening test		Causative agent isolated	Causative agent non-isolated	Mean± S.D.	Coefficient of variation (C.V.: %)
Rolling ball viscosity test (RBVT, Somatic cell×10^6/ml)	~0.49	24(17.9)	21(58.3)		
	0.50~1.00	31(23.1)	7(19.4)		
	1.01~1.50	6(4.5)			
	1.51~2.00	15(11.2)	1(2.8)		
	2.01~2.50	3(2.2)	1(2.8)		
	2.51~	55(41.0)	6(16.7)		
	Total	134(100.0)	36(100.0)	1.620± 1.167	72.0
California mastitis test (CMT)*	−	3(2.2)	13(36.1)		
	±	1(0.7)			
	1+	29(21.6)	9(25.0)		
	2+	44(32.8)	7(19.4)		
	3+	57(42.5)	7(19.4)		
	Total	134(100.0)	36(100.0)	2.9± 1.2	41.4
Whiteside test(WT)*	−	2(1.5)	13(36.1)		
	±	1(0.7)			
	1+	38(28.4)	10(27.8)		
	2+	41(30.6)	7(19.4)		
	3+	52(38.8)	6(16.7)		
	Total	134(100.0)	36(100.0)	2.8± 1.2	42.9

* Reaction values were calculated as follows: negative=0, ± =1, 1+ =2, 2+ =3, and 3+ =4 points respectively.

Table 3. Distribution of comparative relation among rolling ball viscosity test, california mastitis test and whiteside test

Screening test	Sample examined of 170		Causative agent isolated of 134
	Correlation coefficient	F-test	F-test
Relation between rolling ball viscosity test(Somatic cell×10^6/ml) and california mastitis test	0.82 (P<0.001)	142.39 (P<0.001)	113.95 (P<0.001)
Relation between rolling ball viscosity test(Somatic cell×10^6/ml) and whiteside test	0.75 (P<0.001)	98.87 (P<0.001)	77.47 (P<0.001)
Relation between california mastitis test and whiteside test	0.93 (P<0.001)	4.67 (P<0.5)	3.74 (P>0.5)

Table 4. Screening tests of raw milk of quarters in bovine mastitis by criteria of rolling viscosity test(%)

Causative agent isolated

Rolling ball viscosity test (RBVT; Somatic cell $\times 10^6$/ml;	No. of sample)	California mastitis test(CMT)					whiteside test(WT)				
		−	±	1+	2+	3+	−	±	1+	2+	3+
~0.49	24 (17.9)		1 (100.0)	21 (72.4)	2 (4.5)			1 (100.0)	16 (42.1)	7 (17.1)	
0.50~1.00	31 (23.1)	3 (100.0)		8 (27.6)	20 (45.5)				11 (28.9)	20 (48.8)	
1.01~1.50	6 (4.5)				4 (9.1)	2 (3.5)			1 (2.6)	3 (7.3)	2 (3.8)
1.51~2.00	15 (11.2)				10 (22.7)	5 (8.8)			5 (13.2)	8 (19.5)	2 (3.8)
2.01~2.50	3 (2.2)				1 (2.3)	2 (3.5)	2 (100.0)			1 (2.4)	
2.51~3.00	6 (4.5)					6 (10.5)			4 (10.5)	2 (4.9)	
3.01~	49 (36.6)				7 (15.9)	42 (73.7)			1 (2.6)		48 (92.3)
Total	134 (100.0)	3 (100.0)	1 (100.0)	29 (100.0)	44 (100.0)	57 (100.0)	2 (100.0)	1 (100.0)	38 (100.0)	41 (100.0)	52 (100.0)

Causative agent non-isolated

Rolling ball viscosity test (RBVT; Somatic cell $\times 10^6$/ml;	No. of sample)	California mastitis test(CMT)					whiteside test(WT)				
		−	±	1+	2+	3+	−	±	1+	2+	3+
~0.49	21 (58.3)	12 (92.3)		7 (77.8)	1 (14.3)	1 (14.3)	12 (92.3)		7 (70.0)	1 (14.3)	1 (16.7)
0.50~1.00	7 (19.4)	1 (7.7)		2 (22.2)	4 (57.1)		1 (7.7)		2 (20.0)	4 (57.1)	
1.01~1.50											
1.51~2.00	1 (2.8)				1 (14.3)					1 (14.3)	
2.01~2.50	1 (2.8)					1 (14.3)					1 (16.7)
2.51~3.00	1 (2.8)					1 (14.3)					1 (16.7)
3.01~	5 (13.9)				1 (14.3)	4 (57.1)			1 (10.0)	1 (14.3)	3 (50.0)
Total	36 (100.0)	13 (100.0)		9 (100.0)	7 (100.0)	7 (100.0)	13 (100.0)		10 (100.0)	7 (100.0)	6 (100.0)

Table 5. Monthly causative agents isolated from raw milk of quarters in bovine mastitis by screening tests(%)

Screening test / Month (No. of sample)		Rolling ball viscosity(RBVT; Somatic cell×10^6 ml)							California mastitis test(CMT)					Whiteside test(WT)				
		~0.49	0.50~1.00	1.01~1.50	1.51~2.00	2.01~2.50	2.51~3.00	3.01~	−	±	1+	2+	3+	−	±	1+	2+	3+
Jan.	12 (9.0)	4 (16.7)	3 (9.7)		1 (6.7)	1 (33.3)	1 (16.7)	2 (4.1)	3 (100.0)	1 (100.0)	4 (13.8)	2 (4.5)	2 (3.5)	2 (100.0)	1 (100.0)	5 (13.2)	1 (2.4)	3 (5.8)
Feb.	10 (7.5)	4 (16.7)	1 (3.2)				1 (16.7)	4 (8.2)			2 (6.9)	2 (4.5)	6 (10.6)			4 (10.5)	2 (4.9)	4 (7.7)
Mar.	9 (6.7)	1 (4.2)	2 (6.5)	2 (33.3)	1 (6.7)		1 (16.7)	2 (4.1)			1 (3.4)	3 (6.8)	5 (8.8)			1 (2.6)	4 (9.8)	4 (7.7)
Apr.	11 (8.2)	3 (12.5)	4 (12.9)		1 (6.7)		1 (16.7)	2 (4.1)			1 (17.2)	3 (6.8)	3 (5.3)			1 (10.5)	4 (9.8)	3 (5.8)
May	6 (4.5)		2 (6.5)		1 (6.7)			3 (6.1)				3 (6.8)	3 (5.3)				3 (7.3)	3 (5.8)
Jun.	15 (11.2)	1 (4.2)	1 (3.2)	1 (16.7)	2 (13.3)			10 (20.4)			1 (3.4)	3 (6.8)	11 (19.3)			1 (2.6)	5 (12.2)	9 (17.3)
Jul.	17 (12.7)	2 (8.3)	4 (12.9)		4 (26.7)			7 (14.3)			2 (6.9)	6 (13.6)	9 (15.8)			2 (5.3)	3 (7.3)	12 (23.1)
Aug.	24 (17.9)	5 (20.8)	3 (9.7)	2 (33.3)	1 (6.7)	2 (66.7)		11 (22.4)			6 (20.7)	8 (18.2)	10 (17.5)			6 (15.8)	8 (19.5)	10 (19.2)
Sep.	22 (16.4)	2 (8.3)	9 (29.0)		2 (13.3)		1 (16.7)	8 (16.3)			6 (20.7)	8 (18.2)	8 (14.0)			10 (26.3)	8 (19.5)	4 (7.7)
Oct.	4 (3.0)	2 (8.3)	2 (6.5)								2 (6.9)	2 (4.5)				2 (5.3)	2 (4.9)	
Nov.	3 (2.2)			1 (16.7)	1 (6.7)		1 (16.7)					3 (6.8)				3 (7.9)		
Dec.	1 (0.7)				1 (6.7)							1 (2.3)					1 (2.4)	
Total	134 (100.0)	24 (100.0)	31 (100.0)	6 (100.0)	15 (100.0)	3 (100.0)	6 (100.0)	49 (100.0)	3 (100.0)	1 (100.0)	29 (100.0)	44 (100.0)	57 (100.0)	2 (100.0)	1 (100.0)	38 (100.0)	41 (100.0)	52 (100.0)

Table 6. Causative agents isolated from milk of quarters in bovine mastitis by screening tests(%)

Caustative agent(No. of sample)	Screening test		Rolling ball viscosity test(RBVT: Somatic cell×10^6/ml)							California mastitis test(CMT)					Whiteside test(WT)				
			~0.49	0.50~1.00	1.01~1.50	1.51~2.00	2.01~2.50	2.51~3.00	3.01~	−	±	1+	2+	3+	−	±	1+	2+	3+
Streptococcus sp.	9 (6.7)		1 (4.2)	3 (9.7)		1 (6.7)			4 (8.2)			1 (3.4)	3 (6.8)	5 (8.8)			2 (5.3)	4 (9.8)	3 (5.8)
Staphylococcus aureus	1 (0.7)	(51.4)							1 (2.0)					1 (1.8)					1 (1.9)
Staphylococcus epidermidis	1 (0.7)				1 (16.7)								1 (2.3)					1 (2.4)	
Staphylococcus sp.	67 (50.0)		16 (66.7)	18 (58.1)	1 (16.7)	5 (33.3)	2 (66.7)	5 (83.3)	20 (40.8)	3 (100.0)	1 (100.0)	20 (69.0)	18 (40.9)	25 (43.9)	2 (100.0)	1 (100.0)	23 (60.5)	16 (39.0)	25 (48.1)
Escherichia coli	32 (23.9)		7 (19.2)	4 (12.9)	3 (50.0)	4 (26.7)		1 (16.7)	13 (26.5)			6 (20.7)	12 (27.3)	14 (24.6)			10 (26.3)	9 (22.0)	13 (25.0)
Enterobacteriaceae	1 (0.7)						1 (33.3)						1 (2.3)					1 (2.4)	
Klebsiella sp.	1 (0.7)								1 (2.0)					1 (1.8)				1 (2.4)	
Proteus sp.	1 (0.7)					1 (6.7)							1 (2.3)					1 (2.4)	
Pseudononas sp.	15 (11.2)			5 (16.1)	1 (16.7)	2 (13.3)			7 (14.3)			2 (6.9)	6 (13.6)	7 (12.3)			2 (5.3)	6 (14.6)	7 (13.5)
Salmonella sp.	2 (1.5)					2 (13.3)							1 (2.3)	1 (1.8)			1 (2.6)	1 (2.4)	
Corynebacterium sp.	4 (3.0)			1 (3.2)					3 (6.1)				1 (2.3)	3 (5.3)				1 (2.4)	3 (5.8)
Total	134 (100.0)		24 (100.0)	31 (100.0)	6 (100.0)	15 (100.0)	3 (100.0)	6 (100.0)	49 (100.0)	3 (100.0)	1 (100.0)	29 (100.0)	44 (100.0)	57 (100.0)	2 (100.0)	1 (100.0)	38 (100.0)	41 (100.0)	52 (100.0)

Table 7. Antimicrobial susceptibility of causative agents isolated from raw milk of quarters of 134 in bovine mastitis(%).

Causative agent / Antimicrobia	*Streptococcus* sp.	*Staphylococcus aureus*	*Staphylococcus epidermidis*	*Staphylococcus* sp.	*Escherichia coli*	*Enterobacteriaceae*	*Klebsiella* sp.	*Proteus* sp.	*Pseudomonas* sp.	*Salmonella* sp.	*Corynebacterium* sp.
Total	9(100.0)	1(100.0)	1(100.0)	67(100.0)	32(100.0)	1(100.0)	1(100.0)	1(100.0)	15(100.0)	2(100.0)	4(100.0)
Ampicillin(AM)	6(66.7)			33(49.3)	10(31.3)				3(20.0)	1(50.0)	3(75.0)
Cephalothin(CF)	5(55.6)	1(100.0)	1(100.0)	42(62.7)	16(50.0)				7(46.7)	1(50.0)	2(50.0)
Chloxacillin(CX)	4(44.4)		1(100.0)	21(31.3)	9(28.1)				1(6.7)	1(50.0)	
Enrofloxacin(ENR)	7(77.8)	1(100.0)	1(100.0)	55(82.1)	26(81.3)	1(100.0)	1(100.0)	1(100.0)	11(73.3)	2(100.0)	3(75.0)
Erythromycin(EM)	2(22.2)		1(100.0)	29(43.3)	10(31.3)				1(6.7)	1(50.0)	3(75.0)
Gentamycin(GM)	7(77.8)	1(100.0)		48(71.6)	22(68.8)	1(100.0)	1(100.0)	1(100.0)	6(40.0)	2(100.0)	2(50.0)
Kanamycin(KM)	3(33.3)	1(100.0)		39(58.2)	19(59.4)	1(100.0)		1(100.0)	1(6.7)	1(50.0)	3(75.0)
Lincomycin(LM)	1(11.1)			4(6.0)	2(6.3)				1(6.7)	1(50.0)	1(25.0)
Neomycin(NM)	4(44.4)	1(100.0)	1(100.0)	38(56.7)	20(62.5)	1(100.0)	1(100.0)		2(13.3)	2(100.0)	1(25.0)
Penicillin(PP)	3(33.3)		1(100.0)	26(38.8)	3(9.4)				2(13.3)		2(50.0)
Streptomycin(SM)	2(22.2)			18(26.9)	10(31.3)				2(13.3)	2(100.0)	1(25.0)
Tetracycline(TE)	6(66.7)		1(100.0)	33(49.3)	14(43.8)			1(100.0)	2(13.3)		3(75.0)
Trimethoprim/ Sulfamethoxazole(SXT)	7(77.8)		1(100.0)	44(65.7)	17(53.1)			1(100.0)	7(46.7)	2(100.0)	2(50.0)

relation between california mastitis test and whiteside test in this estimation as indicated by the F-test statistic was observed in error due to interval of large reaction values of rolling ball viscosity test (somatic cell$\times 10^6$ per milliliter) generally (Table 3). The reaction values of somatic cell per milliliter were observed in the order of over than 3.01×10^6(36.6%), 0.50×10^6-1.00×10^6(23.1%), less than 0.49×10^6(17.9%) in the causative agents isolated from quarters of mastitis, but less than 0.49 $\times 10^6$(58.3%), 0.50×10^6-1.00×10^6(19.4%) over than 3.01×10^6(13.9%) in the causative agents non-isolated in Table 4.

The reaction values of both california mastitis test(CMT) and whiteside test(WT) by criteria of rolling ball viscosity test(RBVT: somatic cell per milliliter) in the causative agents isolated from quarters of mastitis were observed in the high degree of 1+(CMT: 72.4%, WT: 42.1%) in somatic cell of less than 0.49×10^6, 2+(CMT: 45.5%, WT: 48.8%) in 0.50×10^6-1.00×10^6, 3+(CMT: 73.7%, WT: 92.3%) in over than 3.01×10^6, but in the non-isolated causative agent were observed in the high degree of negative (both CMT and WT: 92.3 %) in less than 0.49×10^6, 1+(CMT; 77.8%, WT; 70.0%) in less than 0.49×10^6, 3+(CMT; 57.1%, WT; 50.0%) in over than 3.01×10^6 respectively (Table 4). The occurrence frequencies by season of causative agents isolated from quarters of mastitis were observed in the order of August(17.9%), September (16.4%), July(12.7%), June(11.2%), and January(9.0%) etc. in Table 5. The screening tests by season in the causative agents isolated from quarters of mastitis were observed in highest of August(20.8%) of less than 0.49×10^6, September (29.0%) of 0.50×10^6-1.00×10^6, both March and August(33.3%) of 1.01×10^6-1.50×10^6, July (26.7%) of 1.51×10^6-2.00×10^6, August(66, 7%) of 2.01×10^6-2.50×10^6, August(22.4%) of over than 3.01×10^6 in somatic cell per milliliter, and then both August and September (18.2%) of 2+, June(19.3%) of 3+ in california mastitis test, and in addition to both August and September(19.5%) in 2+, July (23.1%) of 3+ in whiteside test generally (Table 5).

The frequencies of isolation of mastitis causing pathogens from quarters were observed in the order of *Staphylococus* sp. (51.4%), *Escherichia coli* (23.9%), *Pseudomonas* sp.(11.2%), and *Streptococcus* sp. (6.7%) in Table 6. The frequencies of isolation of mastitis causing pathogens from quarters by screening tests were observed in the high degree of *Staphylococcus* sp., and *Escherichia coli* generally (Table 6). The susceptibility of antimicorbias to the causative agents was observed in the highest of enrofloxacin(ENR), gentamycin(GM), and trimethoprim/sulfamethoxazole(SXT) of each 77.8% to *Streptococcus* sp., cephalothin(CF), enrofloxacin (ENR), gentamycin(GM), kanamycin(KM), and neomycin(NM) of each 100.0% to *Staphylococcus auteus*, cephalothin(CF), chloxacillin(CX), enrofloxacin(ENR), erythromycin(EM), neomycin(NM), penicillin(PP), kanamycin(KM), tetracyclin(TE), and trimethoprim/sulfamethoxazole(SXT) of each 100.0% to *Staphylococcus epidermidis*, enrofloxacin (ENR: 82.1%) to *Staphylococcus* sp., enrofloxacin (ENR: 81.3%) to *Escherichia coli*, enrofloxacin (ENR), gentamycin(GM), kanamycin(KM), and neomycin(NM) of each 100.0% to *Enterobacteriaceae*, enrofloxacin(ENR), gentamycin(GM), and neomycin(NM) of each 100.0% to *Klebsiella* sp., enrofloxacin(ENR), gentamycin(GM), tetracycline (TE), and trimethoprim/sulfamethoxazole(SXT) of each 100.0% to *Proteus* sp., enrofloxacin(ENR: 73.3 %) to *Psedomonas* sp., enrofloxacin(ENR), gentamycin(GM), neomycin(NM), streptomycin(SM), and trimethoprim/sulfamethoxazole(SXT) of each 100.0 % to *Salmonella* sp., and then ampicillin to *Corynebacterium* sp. in Table 7.

Discussion

In this study, the outbreaks of bovine mastitis by examining of raw milk was observed in subclinical cows of 323(20.0%), clinical cows of 70 (4.3%) among the feeding dairy cows of 1,614 on dairy farms, and then subclinical quarters of 643 (10.0%), clinical quarters of 87(1.3%) among the quarters of 6,456. It has been estimated that subclinical mastitis occurs in 28 percent of quarters and in 55 percent of cows(Dodd and Jackson, 1971) by Linzell[13]. Na and Kang(1975) reported that clinical mastitis was found at quarters of 7(3.5 %) in cows of 5(5.0%).[14] Kim(1974) reported that milk samples from 1,231(38.1%) of quarters of 3, 225 and 568(69.3%) of dairy cattle of 820 were positive for mastitis by california mastitis test.[15] Son(1974) reported that a total of quarters of 428

(20.8%) of dairy cows of 271(52.3%) from herds of 41(89%) were found to be infected with mastitis. And also. it was found that quarters of 71(3.5 %) of cows of 41(7.9%) from herds of 21(45.6%) were clinical mastitis, and quarters of 357(17.3%) of cows of 230(44.4%) from herds fo 20(43.4%) were subclinical mastitis.[16] Song(1975) reported that quarters of 654(31.6%) of dairy cows of 301 (56.3%) from herds of 34(97.1%) were infected with mastitis. It was found that cows of 8(1.5%) showed clinical mastitis and other cows were subclinical.[17]

Jung(1994) reported that 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 in Chonnam area.[5] As the result, it was observed in apparent difference by region, year, sample and symptom of case. In this study, the causative agents were isolated from raw milk of quarters of 134(18.4%) among the quarters of 730. Kitchen(1984) reported that a lower proportion than this author indicates in the major pathogens found in 12.5%.[18] As the result, they were observed in apparent difference by region, year, grouping of major pathogen and sample.

In this study, the mean of somatic cell number was observed in $1.620\times10^6\pm1.167\times10^6$ per milliliter(C.V.; 72.0%) by rolling ball viscosity test (RBVT). Result of this study indicated that reaction values are in higher mean than somatic cell counts of $1.059\times10^6\pm0.795\times10^6$ per milliliter in a rural area by Jung(1994).[5] The above result was observed in apparent difference by region, year, and symptom of case in consequence. Schalm (1977) reported that somatic cell numbers in foremilk from healthy bovine quarters are commonly less than 100×10^3 per milliliter.[19]

Erskine(1983) reported that herds selected for the study had somatic cell counts of less than half or more than twice the statewide mean 12-month somatic cell counts for Pennsylvania Dairy Herd Improvement Association herds(ie, 329×10^3 cell per milliliter).[20,21] And Schukken(1993) reported that standard deviation in the prediction error was still approximately 150×10^3 cells (somatic cell counts per milliliter).[22]

In this study, the arithemtic mean of reaction values was observed in 2.9 ± 1.2(C.V.: 41.4%) by california mastitis test(CMT), and 2.8 ± 1.2(C.V.; 42.9%) by whiteside test(WT). Result of this study indicated that reaction values are in higher mean than modified whiteside test of 1.09 ± 0.01, modified california mastits test of 1.12 ± 0.06, and resazurin reduction test of 1.25 ± 0.40 by Na(1975).[23] As the result, they were observed in apparent difference by region, year and test method.

In this study, the correlation coefficient was distributed in 0.82($P<0.001$) by relation between rolling ball viscosity test (somatic cell$\times10^6$ per milliliter) and california mastitis test. 0.75($P<0.001$) by relation between rolling ball viscosity test(somatic cell$\times10^6$ per milliliter) and whiteside test, and then 0.93($P<0.001$) by relation between california mastitis test and whiteside test.

However, significant comparison of excepting relation between california mastitis test and whiteside test in this estmation as indicated by the F-test statistic was observed in error due to interval of large reaction value of rolling ball viscosity test (somatic cell$\times10^6$ per milliliter) generally.

The reaction values of somatic cell per milliliter were observed in the order of over than 3.01×10^6 (36.6%), 0.50×10^6-1.00×10^6(23.1%), less than 0.49×10^6(17.9%) in the causative agents isolated from quarters of mastitis, but less than 0.49×10^6(58.3%), 0.50×10^6-1.00×10^6(19.4%), over than 3.01×10^6 (13.9%) in the non-isolated causative agents. And also, the reaction values of both california mastitis test and whiteside test by criteria of rolling ball viscosity test (somatic cell per milliliter) in the causative agents isolated from quarters of mastitis were observed in the high degree of 1+(CMT: 72.4%, WT: 42.1%) in somatic cell of less than 0.49×10^6, 2+(CMT; 45.5%, WT: 48.8%) in 0.50×10^6-1.00×10^6, 3+(CMT; 73.7%, WT: 92.3%) in over than 3.01×10^6, but in the non-isolated causative agents were observed in the high degree of negative (both CMT and WT; 92.3%)in less than 0.49×10^6, 1+(CMT: 77.8%, WT; 70.0%) in less than 0.49×10^6, 3+(CMT; 57.1%, WT; 50.0%) in over than 3.01×10^6 respectively.

The occurrence frequencies by season of causative agents isolated from quarters of mastitis were observed in the order of mastits were observed in the order of August(17.9%), September(16.4%), July(12.7%), June(11.2%), and January(9.0%) etc.

This study showed that some differences due to regions and years were observed in the order of August(17.0%), September(12.7%), April(11.2%), July(10.4%) and October(10.4%) by Jung(1994).[5]

The screening test by season in the causative agents isolated from quarters of mastitis was observed in the highest of August(20.8%) of less than 0.49×10^6, September(29.0%) of 0.50×10^6-1.00×10^6, both March and August(33.3%) of 1.01×10^6-1.50×10^6, July(26.7%) of 1.51×10^6-2.00×10^6, August(66.7%) of 2.01×10^6-2.50×10^6, August(22.4%) of over than 3.01×10^6 in somatic cell per milliter, and then both August and September(18.2%) of 2+, June(19.3%) of 3+ in california mastitis test, and in addition to both August and September (19.5%) in 2+, July(23.1%) of 3+ in whiteside test generally.

The frequencies of isolation of mastitis causing pathogens from quarters were observed in the order of *Staphylococcus* sp. (51.4%), *Escherichia coli* (23.9%), *Pseudomonas* sp. (11.2%), and *Streptococcus* sp. (6.7%) Okigbo(1984) reported that bacterial groups isolated from quarter milk samples were occurred in the order of *Staphylococcus* (coagulase; negative; 50%), *Corynebacterium*(18%), both *Streptococcus* and *Staphylococcus* (coagulase; positive; 7%), coliforms (1%), and none (18%).[24]

Schukken(1988) reported that bacteria isolated from mastitic cows was occurred in the order of *Streptococcus agalactiae*(13.5%), other *Streptococci* (13.1%), *Staphylococcus aureus* (8.3%), *Escherichia coli*(2.4%), and *Klebsiella* sp. (0.5%), but no bacteria isolated(55.0%).[25] Hazlett(1984) reported that numbers diagnosed from mastitis cases were occurred in the order of *Escherichia coli*(41.0%), mixed(2 pathogens; 22.2%), no etiological diagnosis (10.8%), *C. pyogenes*(7.5%), others(1 pathogens; 6.6%), *Klebsiella* sp. (5.7%), *S. aureus* (4.2%), and *Streptococci*(1.9%).[1]

And Jung(1994) reported that bacteria isolated from mastitis cows observed in the order of *Streptococcus* sp. (20.1%), *Escherichia coli*(16.6%), *Staphylococcus aureus*(13.5%), *Salmonella* sp. (11.2%), and *Staphylococcus* sp. (10.8%) etc.[5] The above result was observed in apparent difference by region, year, sample, symptom of case, and test method in consequence. The frequencies of isolation of mastitis causing pathogens from quarters by screening tests were observed in the high degree of *Staphylococcus* sp., and *Escherichia coli* generally.

The susceptibility of antimicrobias to the causative agents was observed in the highest of enrofloxacin(ENR), gentamycin(GM), and trimethoprim/sulfamethoxazole(SXT) of each 77.8% to *Streptococcus* sp., cephalothin(CF), enrofloxacin (ENR), gentamycin(GM), kanamycin(KM), and neomycin(NM) of each 100.0% to *Staphylococus aureus*, cephalothin(CF), chloxacillin(CX), enrofloxacin(ENR), erythromycin(EM), neomycin(NM), penicillin(PP), tetracycline(TE), and trimethoprim/sulfamethoxazole(SXT) of each 100.0% to *Staphylococcus epidermidis*, enrofloxacin(ENR: 82.1%) to *Staphylococcus* sp., enrofloxacin(ENR: 81.3%) to *Escherichia coli*, enrofloxacin(ENR), gentamycin (GM), kanamycin(KM), and neomycin(NM) of each 100.0% to *Enterobacteriaceae*, enrofloxacin(ENR), gentamycin(GM), and neomycin(NM) of each 100.0% to *Klebsiella* sp., enrofloxacin(ENR), gentamycin (GM), kanamycin(KM), tetracycline(TE), and trimethoprim/sulfamethoxazole(SXT) of each 100.0% to *Proteus* sp., enrofloxacin(ENR: 73.3%) to *Pseudomonas* sp., enrofloxacin(ENR), gentamycin(GM), neomycin(NM), streptomycin(SM), and trimethoprim/sulfamethoxazole(SXT) of each 100.0% to *Salmonella* sp., and then ampicillin(AM), enrofloxacin (ENR), erythromycin(EM), kanamycin(KM), and tetracycline(TE) of each 75.0% to *Corynebacterium* sp..

Stem(1984) reported that antimicrobias had been used to mastitis treatments in cephapirin sodium to *Escherichai coli*, *Streptococcus* sp., *Prototheca* sp., yeast, gram positive rod, gentamycin sulfate to *Escherichia coli*, *Streptococcus* sp., and yest, potassium hetacillin to *Escherichia coli*, *Klebsiella* sp., oxytetracycline hydrochloride to *Escherichia coli*, *Staphylococcus* sp., and mold, polymixin B sulfate to *Escherichia coli*, sodium cloxacillin to *Klebsiella* sp., and yest, procaine penicillin G to *Corynebacterium*, *C. Pyogenes*, *Escherichia coli*, *Klebsiella* sp., and gram negative rod, ampicillin trihydrate to gram negative rod, kanamycin sulfate to *Streptococcus* sp., nitrofurazone(0.2% solution) to gram negative rod, iodine mastitis mix(90% mineral oil, 6% ethyl ether, 4% iodine crystals) to *Streptococcus* sp., *C. pyogenes*, and *Escherichia coli*[(26)].

Therefore, the susceptibility of antimicrobias to the causative agents was observed in the highest of trimethoprim/sulfamethoxazole to *Streptococcus* sp., *Staphylococcus epidermidis, Proteus* sp., *Salmonella* sp., gentamycin to *Streptococcus* sp., *Staphylococcus aureus, Enterobacteriaceae, Klebsiella* sp., *Proteus* sp., and *Salmonella* sp., and then enrofloxacin to almost agents in general. As the result, it was obseved in apparent difference by region, year, strain and kind of antimicrobia.

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