

Assessment of Immune Quality and Pathogen Contamination of Colostrums Collected from Colostrum Banks in Korea

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Abstract : Because colostrum is considered to be the sole source of passively acquired maternal antibodies for calves, newborn calves must consume colostrum to gain disease resistance during their early years of life. Storage of surplus colostrum from dairy cows right after calving and feeding newborn calves in deficiency of colostrum to assure adequate uptake of IgG for protection of the calf has been a common practice in the bovine production. In the current study, 35 colostrums were randomly collected from 3 colostrum banks located in different regions of Korea and monitored for general bacterial contamination and major bovine pathogens. Immunoglobulin concentrations and BVDV-specific antibodies were also determined to evaluate the immune quality of the colostrums. Moderate to severe bacterial contamination (up to 72,000,000 CFU/ml) was observed in most of the colostrums collected from colostrum banks. General immune quality of the colostrums was under the satisfactory level since most of the colostrums contained less than 50 g/L of IgG, which is the minimum concentration for good quality colostrums. Therefore, colostrum for colostrum bank should be collected at the first 2-3 post-partum milkings according to proper harvesting and handling procedures to guarantee the safety and quality of colostrum. In addition, it was recommended that colostrum should be heat-treated before frozen and stored in the bank because pasteurization at 63°C for 30 min was very effective reducing the risk of disease transmission without causing significant degradation of immunoglobulins.

Key words : bovine colostrum, colostrum bank, bacterial contamination, bovine pathogens, immunoglobulins.

Introduction

It has been reported that gastrointestinal disorder is the most frequent diagnosis in calves and caused the most economically significant problem in Korean cattle farms (1). Although various pathogens, such as bovine coronavirus (BCoV), bovine rotavirus (BRV), bovine viral diarrhea virus (BVDV), *Salmonella* spp., *E. coli* K99⁺, *Clostridium* spp, *Cryptosporidium* and *Coccidium* could be major causative factors in calf diarrhea, insufficient uptake of colostrum is one of the most important factor (2-4). If new born calves can be supplied with enough amount of colostrum in the first 1-2 days (24hours) after birth, it can dramatically reduce the morbidity and mortality of diarrhea (5). Colostrum banks that collect and save surplus colostrum from neighboring dairy farms and provide colostrum for hanwoo farms where colostrum is insufficient to feed new born calves have been operated in many Agricultural Technique Centers (ATC) in Korea.

The biggest problem in this system, however, is disease transmission because various pathogens can be contaminated in colostrum and it could be the major source for disease

spread between farms. In fact, bovine pathogens such as *Mycobacterium bovis* (6), bovine viral diarrhea virus (BVDV) (7) have been detected in colostrum and colostrum can be accidentally contaminated with various bacteria during colostrum harvesting (8). In addition, the quality of colostrum is important to achieve a good level of protection against various diseases related to new born mortality. In general, new born calves need to consume at least 2-3 liters of colostrum containing 70 g/L of IgG within 6 hr after birth for good protection (5). Therefore, in current study, 35 colostrums were randomly collected from 3 colostrum banks located in different regions of Korea and assessed for the presence of general bacterial contamination and major bovine pathogens such as BCoV, BRV, BVDV, *Salmonella* spp., *E. coli* K99⁺, *M. bovis*, *M. tuberculosis*, and *Cryptosporidium*. Immunoglobulin concentrations and BVDV-specific antibodies in the collected colostrums also were determined to evaluate the immune quality of the colostrums.

Materials and methods

Colostrums

A total of 35 colostrums were randomly collected from 3 colostrum banks in ATCs located in 3 different regions. The colostrums have been delivered frozen to the laboratory and

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kept in -20°C until used. Then the colostrums were thawed as quickly as possible in a water bath set at 37°C for evaluation.

Bacterial culture

Each colostrum was serially diluted from 10^1 to 10^6 with 0.01 M PBS (pH = 7.4). Then 200 μl of the diluted colostrum was inoculated on LB (USB Corporation, USA) and incubated for 16 hr at 37°C . After incubation, colony forming unit per ml (CFU/ml) was determined by counting the number of viable bacterial colonies from a plate containing less than 50 colonies.

Nucleic acid extraction

Twenty-five ml of colostrum was centrifuged at $1000 \times g$ for 15 min at 4°C to pellet somatic cells and the supernatant was discarded. The cell pellet was resuspended in 15 ml of 0.01 M PBS and centrifuged at $200 \times g$ for 15 min at 4°C . The final cell pellet was resuspended in 0.5 ml of PBS and stored in -80°C until used. Total nucleic acids were extracted

from colostrums by use of MagMaxTM Total Nucleic Acid Isolation Kit (Applied Biosystems, USA) as described in the manufacturer's manual. Briefly, 175 μl of each cell pellet was added to a tube containing 235 μl of lysis/binding solution. The bead tube was beaten at maximum speed for 5 min with Bullet Blender[®] (Next Advance, USA). After the beating process, the bead tubes were centrifuged at $16,000 \times g$ for 3 min, and the supernatant was carefully transferred into clean microcentrifuge tubes. After another centrifugation at $16,000 \times g$ for 6 min, 115 μl of the supernatant was transferred to a 96-well microplate along with washing and elution buffers as suggested by manufacturer's manual. The automated process consisted of lysis/binding step for 5 min; two-time first washing step each for 90 sec; two-time second washing step each for 2 min and 30 sec respectively; dry step for 1 min; and, finally, the elution step for 3 min. The extracted total nucleic acids in the elution plate were stored in -80°C until used for PCR reaction.

Table 1. The primers or probes information for PCR tests used in the current study

Agent (target gene)	Primer or probe (5'/3' labels)	Sequence (5'-3')	Product size (bp)	Reference no.
BVDV (M)	BVDV-F (fwd)	GGGNAGTCGTCARTGGITTCG	190	9
	BVDV-R (rev)	GTGCCATGTACAGCAGAGWTTTT		
	BVDV probe (Cy5/BHQ2)	CCAYGTGGACGAGGGCAYGC		
BCoV (N)	BCoV-fwd	CTAGTAACCAGGCTGATGTCAATACC	87	9
	BCoV-rev	GGCGGAAACCTAGTCGGAATA		
	BCoV-probe (FAM/MGB)	CGGCTGACATTCTCGATC		
BRV group A (VP6)	BRV-fwd1	TCAACATGGATGTCCTGTACTCCT	155	9
	BRV-fwd2	TCAACATGGATGTCCTGTATTTCCT		
	BRV-fwd3	TCAACATGGATGTCCTTTATTTCCT		
	BRV-rev1	TCCTCCAGTTTGGAATTCATT		
	BRV-rev2	TCCCCAGTTTGGAATTCATT		
	BRV-rev3	CCCTCCAGTTTGGAATTCATT		
<i>E. coli</i> K99 ⁺ (K99)	BRV-probe1 (VIC/MGB)	TCAAAAACCTCTTAAAGATGCTAG	80	9
	BRV-probe2 (VIC/MGB)	TCAAAAACCTCTTAAAGATGCAAG		
	K99-fwd	GCTATTAGTGGTCATGGCACTGTAG		
<i>Salmonella</i> (Stn)	K99-rev	TTTGTTTTCGCTAGGCAGTCATTA	129	9
	K99-Probe (FAM/BHQ)	ATTTTAAACTAAAACCAGCGCCCGCA		
	Stn-fwd	GCCATGCTGTTTCGATGAT		
<i>Cryptosporidium</i> (COWP)	Stn-rev	GTTACCGATAGCGGAAAGG	151	9
	Stn-probe (Cy5/BHQ)	TTTTGCACCACMGCCAGCCC		
	Crypto-fwd	CAAATTGATACCGTTTGCCTTCTG T		
<i>M. tuberculosis</i> (<i>hupB</i>)	Crypto-rev	GGCATGTCGATTCTAATTCAGCT	645	10
	Crypto-probe (JOE/BHQ)	TGCCATACATTGTTGTCCTGACAAATTGAA		
	N	GAGGGTTGGGATGAACAAAGCAG		
	S	TATCCGTGTGCTTGACCTATTTG		
	F	CCAAGAAGGCGACAAAGG	116	
	R	GACAGCTTTCTTGCGGG		

Real-time PCR panel for detecting bovine enteric pathogens

The sequence information of primers and probes used in the multiplex real-time PCR panel (9) are summarized in Table 1. The multiplex PCR panel was optimized with AgPath-ID™ Multiplex RT-PCR Kit (Applied Biosystems) following manufacturer's recommended protocol in a 25- μ l reaction volume using 8 μ l of extracted template. All primers and probes were prepared at 25- μ M working concentration, and equal volumes of primers and probes were mixed together for each target agent. Two primers and one probe were mixed in a single tube for BVDV, BCoV, *E. coli* K99⁺, *Salmonella*, or *Cryptosporidium* while three each of forward and reverse primers and two probes were mixed together for group A BRV. Two PCR reactions were prepared: one for viral agents (BVDV, BCoV and BRV) and the other for bacterial/protozoan agents (*E. coli* K99⁺, *Salmonella*, and *Cryptosporidium*). The final concentration of each primer or probe was 0.2 μ M. The PCR amplification was performed on the ABI 7500 Fast Real-Time PCR System. Cycling conditions were as follows: a) reverse transcription for 10 min at 45°C (This step was omitted for bacterial/protozoan PCR); b) a 10-min activation step at 95°C; and c) 35 cycles of 15 sec at 95°C and 60 sec at 60°C. Samples with a threshold cycle (Ct) of 35 cycles or less were considered positive. For quantification, a series of positive controls with known virus titer or bacteria number was used to generate a standard curve for each target pathogen. The amount of pathogen detected in colostrum was calculated by converting Ct value to virus titer (TCID₅₀/ml) or bacteria count (CFU/ml) using the standard curve.

PCR for detecting *Mycobacterium bovis* and *Mycobacterium tuberculosis*

PCR was performed to detect *M. bovis* and *M. tuberculosis* with primers listed in Table 1 (10). The PCR was performed with AccPower® Multiplex PCR PreMix Kit (Bioneer, Korea) in a 25- μ l reaction volume using 5- μ l extracted template. The first and second PCR amplification condition was as follows: 95°C for 10 min, and 35 cycles of 95°C for 30 sec, 60°C for 30 sec, and 72°C for 60 sec.

Measurement of total IgG and IgA concentrations in colostrum

The concentrations of IgG and IgA in the colostrums were measured by commercial bovine IgG and IgA ELISA Kits (Bethyl, USA) following the procedures recommended by the manufacturer.

Measurements of antibody against bovine viral diarrhoea virus in colostrums

BVDV-specific antibodies were measured by VDPPro® BVDV Antibody ELISA kit (Median, Korea) following the procedures recommended by the manufacturer. Optical density (OD) was measured at 450 nm and Sample to Negative (SN) ratio was calculated by "sample OD/negative control

OD". SN ratio equal or less than 0.7 was considered as positive.

Results

Detection of IgG and IgA in colostrums from colostrum banks

The levels of IgG and IgA varied greatly among the colostrums. The IgG concentrations of the colostrums ranged from 8.57 to 96.74 g/L while IgA concentration ranged from 0.98 to 31.18 g/L. Only 16 out of 35 colostrums contained equal or higher than 30 g/L of IgG, which is the minimum concentration for adequate quality colostrums and only 10 were considered good quality colostrums which contained equal or higher than 50 g/L of IgG. IgG concentration was positively correlated with IgA in the same colostrums and the correlation was statistically significant ($R^2=0.58$, $p<0.0001$) (Fig 1). BVDV-specific antibody was detected only from 5 colostrums, indicating that most of the colostrums were not collected from cows frequently exposed to BVDV infection.

General bacterial counts on colostrums from colostrum banks

The number of general bacterial cells contaminated in the collected colostrums was determined after general bacterial culture on LB agar plates. A great deal of bacterial colonies was observed from most of the colostrums (up to 72×10^7 CFU/ml). Only 6 colostrums out of 35 were contaminated with less than 2×10^4 CFU/ml, which is the legal limit for bacterial numbers in pasteurized milk (Table 1).

Detection of major bovine pathogens on colostrums from colostrum banks

The presence of BCoV, BRV, BVDV, *Salmonella* spp, *E. coli* K99⁺, *M. bovis*, *M. tuberculosis*, and *Cryptosporidium* was also tested by multiplex PCR and single PCR methods (Table 1). No colostrum was positive for BCoV, BRV, *E. coli*

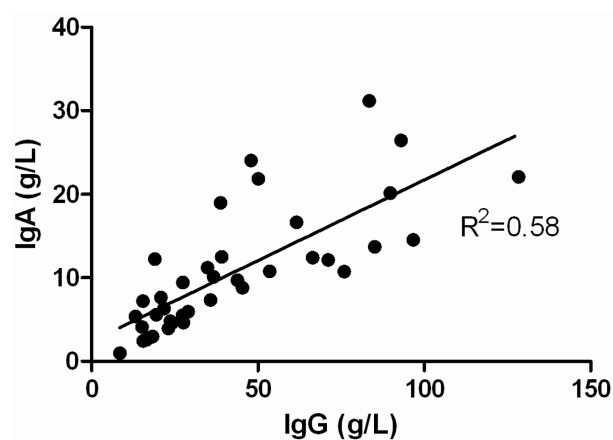


Fig 1. The correlation between IgG and IgA concentrations determined from 35 colostrums collected from 3 colostrum banks operated in Agricultural Technique Centers.

K99⁺, *M. bovis*, *M. tuberculosis*, and *Cryptosporidium* while 14 colostrums were positive for ranging between 6.72×10^1 and 3.10×10^4 CFU/ml, and 1 colostrum was positive for BVDV (8.17×10^3 TCID₅₀/ml)

Effect of pasteurization on colostrums from colostrum banks

To evaluate the effect of pasteurization on the colostrums, all of the colostrums were incubated at 63°C for 30 min in a water bath. The number of bacterial cells was dramatically decreased by approximately 10^4 CFU/ml after pasteurization and the average number of bacterial cells counted from general bacterial culture after pasteurization was less than 100 CFU/ml (Fig 2). IgG and IgA concentrations were also determined after pasteurization to evaluate the destructive effect of pasteurization on immunoglobulin: IgG concentration was decreased by 7.54 ± 4.53 (mean \pm SEM) g/L in average while IgA was decreased by 4.21 ± 1.23 g/L after

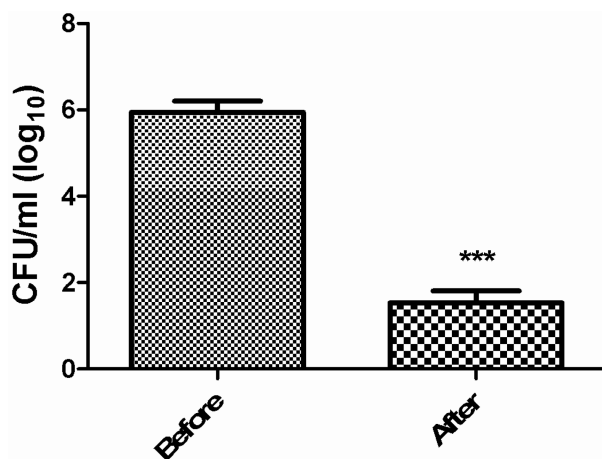


Fig 2. The number of bacterial cells counted from general bacterial culture on 35 colostrums collected from 3 colostrum banks operated in Agricultural Technique Centers before and after pasteurization at 63°C for 30 min. Triple asterisk represents statistical significance ($p < 0.0001$) between before and after pasteurization based on Student's t-test.

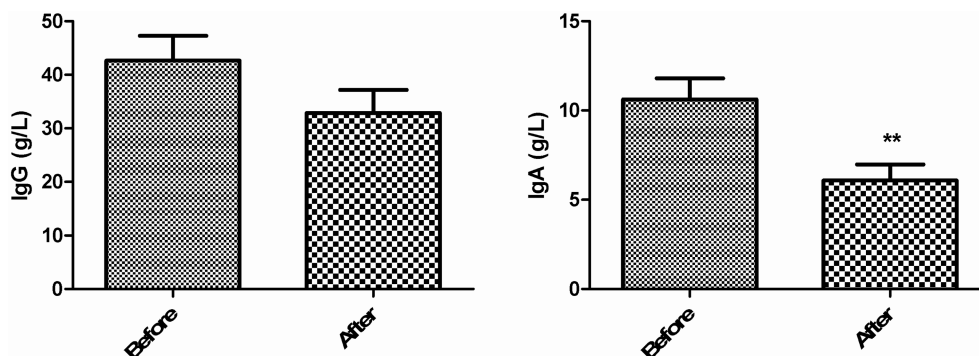


Fig 3. The concentrations of total IgG and IgA of 35 colostrums collected from 3 colostrum banks operated in Agricultural Technique Centers before and after pasteurization at 63°C for 30 min. Double asterisk represents statistical significance ($p < 0.01$) between before and after pasteurization based on Student's t-test.

pasteurization. When IgG or IgA levels before and after pasteurization were analyzed by pair-wise comparison, the decrease of IgA concentration after pasteurization was consistent throughout the collected colostrums and statistically significant ($p < 0.01$). On the other hand, the decrease of IgG concentration after pasteurization was less consistent compared with that of IgA and the difference was not statistically significant ($p > 0.1$) (Fig 3).

Discussion

Because bovine fetus normally receives no passive immunity through the placenta and colostrum is considered to be the sole source of passively acquired maternal antibodies for calves, newborn calves must consume colostrum to gain disease resistance during their early years of life (11). Under optimum conditions, cows will supply more than sufficient colostrum for their calves, but when sufficient colostrum is not available, other sources must be utilized. One possible source of colostrum for emergency use is surplus colostrum from a neighboring dairy. Storage of surplus colostrum from dairy cows right after calving and feeding newborn calves in deficiency of colostrum to assure adequate uptake of IgG for protection of the calf has been a common practice in the bovine production worldwide. This concept has been embodied as colostrum banks established in ATCs in Korea. The biggest concern about sharing surplus colostrum through the colostrum banking system is disease transmission because several pathogens can be transmitted from cow to calf via colostrum (6-8). In the current study, 35 colostrums collected from 3 different colostrum banks were subjected to general bacterial culture and a large number (up to 72×10^7 CFU/ml) of bacterial colonies were observed from most of the colostrums. Only 6 colostrums out of 35 were contaminated with less than 2×10^4 CFU/ml, which is the legal limit for pasteurized milk (Table 1). In addition, 14 colostrums were positive for *Salmonella spp.* ranging between 6.72×10^1 and 3.10×10^4 CFU/ml though other major bovine pathogens such as BCoV, BRV, *E. coli* K99⁺, *M. bovis*, *M. tuberculosis*, and *Cryptosporidium* were not detected from the colostrums by

Table 2. Bacterial or viral pathogens detected from 35 colostrums collected from 3 colostrum banks operated in Agricultural Technique Centers

Colostrum ID	General bacterial culture (CFU ^a /ml)	PCR test	
		<i>Salmonella</i> spp (CFU/ml)	BVDV (TCID ₅₀ /ml)
A1	3,000,000	neg	neg
A2	9,000,000	neg	neg
A3	1,750,000	neg	neg
A4	1,240,000	67.2	neg
A5	11,000,000	neg	neg
A6	21,000,000	146.5	neg
A7	6,000,000	162.3	neg
A8	11,000,000	neg	neg
A9	2,000,000	neg	neg
A10	< 100	138.0	neg
C1	11,000,000	neg	neg
C2	72,000,000	10177.3	neg
C3	12,000,000	5286.7	neg
C4	13,000,000	5319.7	neg
C5	22,000,000	neg	neg
H1	4,300,000	neg	neg
H2	200,000	neg	neg
H3	800,000	neg	neg
H4	300,000	305.6	neg
H5	1,600,000	neg	neg
H6	1,630,000	1140.2	neg
H7	300,000	neg	neg
H8	1,640,000	331.7	8,165
H9	2,700	neg	neg
H10	< 100	neg	neg
H11	960,000	neg	neg
H12	3,200	neg	neg
H13	3,000,000	neg	neg
H14	< 100	neg	neg
H15	170,000	neg	neg
H16	7,200,000	89.2	neg
H17	10,000,000	6716.3	neg
H18	3,000,000	31036.5	neg
H19	1,400,000	28071.6	neg
H20	6,300,000	neg	neg

^a: Colony Forming Unit (CFU).

PCR tests. It has been demonstrated that colostrum may contain these pathogens as a result of shedding from the mammary gland, contamination of the colostrum after harvesting or improper storage of colostrum (8). Thus it was speculated that those pathogens detected in the colostrums tested in the current study were contaminated during harvesting and handling, rather than shed from infected cows since only environ-

Table 3. Antibodies detected from 35 colostrums collected from 3 colostrum banks operated in Agricultural Technique Centers

Colostrum ID	Total IgG (g/L)	Total IgA (g/L)	BVDV blocking ELISA	
			OD ^a	Interpretation*
A1	24.21	4.63	1.12	neg
A2	66.49	12.42	0.59	pos
A3	29.03	5.96	1.16	neg
A4	53.50	10.77	1.17	neg
A5	13.19	5.39	1.11	neg
A6	23.10	3.94	1.11	neg
A7	75.96	10.76	0.57	pos
A8	18.41	2.98	1.09	neg
A9	39.12	12.51	0.76	neg
A10	15.21	4.11	1.00	neg
C1	83.43	31.18	1.05	neg
C2	45.36	8.80	0.92	neg
C3	96.74	14.54	0.96	neg
C4	43.86	9.73	0.93	neg
C5	38.82	18.97	1.05	neg
H1	16.64	2.63	1.08	neg
H2	71.14	12.15	0.62	pos
H3	27.34	5.52	1.12	neg
H4	23.71	4.80	1.15	neg
H5	27.65	4.66	1.12	neg
H6	17.93	2.91	1.07	neg
H7	85.16	13.73	1.11	neg
H8	15.55	2.44	0.65	pos
H9	19.08	12.25	0.79	neg
H10	8.57	0.98	0.99	neg
H11	19.47	5.59	0.82	neg
H12	20.91	7.64	0.96	neg
H13	93.01	26.44	0.33	pos
H14	15.44	7.22	1.04	neg
H15	27.45	9.44	0.81	neg
H16	50.10	21.86	1.03	neg
H17	21.83	6.30	0.97	neg
H18	34.89	11.21	0.76	neg
H19	89.75	20.14	0.94	neg
H20	47.99	24.06	1.04	neg

^a: Optical Density (OD).

*OD value ≤ 0.7 is considered as positive.

mental bacteria including *Salmonella* spp were detected from the colostrum and BVDV-specific antibody was detected only from 5 colostrums indicating that most of the colostrums were not collected from cows frequently exposed to BVDV infection.

Therefore, the colostrum should be pretreated to eliminate the risk of disease transmission before frozen and stored in the bank. Heat-treatment of bovine colostrum has been evaluated

in a previous study (8). Bovine colostrum, inoculated with *M. bovis*, *Listeria monocytogenes*, *E. coli* O157:H7, *Salmonella enteritidis*, and *Mycobacterium avium* subsp. *paratuberculosis* was heat-treated at 60°C for 120 min. It revealed that there was no effect of heating colostrum at 60°C for at least 120 min on mean IgG concentration while viable *M. bovis*, *L. monocytogenes*, *E. coli* O157:H7, and *S. enteritidis* added to colostrum could not be detected. Consistent with this, the number of bacterial cells counted from general bacterial culture was dramatically decreased by 10⁴ CFU/ml to less than 100 CFU/ml and no viable salmonella or BVDV was detected after all of the colostrums were pasteurized at 63°C for 30 min in a water bath in the current study (Fig 2).

Immune quality of the colostrums was also evaluated by measuring immunoglobulin concentrations and pathogen-specific antibodies in the colostrums. Only 16 out of 35 colostrums contained equal or higher than 30 g/L of IgG, which is the minimum concentration for adequate quality colostrums and only 10 were considered good quality colostrums which contained equal or higher than 50 g/L of IgG (5). The quality of colostrum is important to achieve a good level of protection against various diseases related to new born mortality. In general, new born calves need to consume at least 2-3 liters of colostrum containing 70 g/L of IgG within 6 hr after birth for good protection (5). A previous study (8) revealed that the average levels of IgG in the first two post-partum milkings were 60 g/L and the IgG levels in colostrum preserved by freezing were maintained well for the first eight weeks without significant degradation. Colostrum collected at the first milking of the cow after calving contains immunoglobulins which are at their highest concentration and concentrations of immunoglobulins then decline rapidly in the subsequent several milkings (8). Based on these, it was speculated that 25 colostrums which contains less than 50 g/L of IgG were collected too late after calving, rather than stored improperly. Therefore, colostrum for stocking in colostrum bank should be collected at the first 2-3 post-partum milkings to have a good level of IgG concentration (> 50 g/L) in the colostrum.

Because pasteurization of colostrums at 63°C for 30 min is necessary to remove the risk of disease transmission, the effect of pasteurization on immunoglobulins was evaluated in the study. Though both IgG and IgA concentrations were slightly decreased after pasteurization, the decrease of IgG after pasteurization was not statistically significant while the decrease of IgA was statistically significant ($p < 0.01$) based on pair-wise comparison of immunoglobulin concentrations between before and after pasteurization. It has been demonstrated that IgG is the most abundant immunoglobulin class in bovine milk and most thermostable while IgM is the least thermostable (12). Exposure to 75°C can reduce detectable isolated bovine IgG by 40% in 5 min, and by 100% at 95°C for 15 s (13). Heat exposure causes conformational changes in the IgG molecule (14) and reduces antigen-binding activity of bovine IgG (15). IgG in colostrum or colostrum whey also are

reduced by heat treatment, however, at a slower rate than for purified IgG (12). It has been also shown that commercial milk samples that have undergone a typical pasteurization process can retain 25-75% of the IgG concentration compared with raw milk, while milk undergoing ultra-high temperature pasteurization contains little detectable IgG (16).

In conclusion, moderate to heavy bacterial contamination was observed in the colostrums collected from colostrum banks operated in ATC in Korea and the contamination was more likely resulted from improper harvesting and handling. General immune quality of the colostrums was under the satisfactory level since many colostrums contained less than 50 g/L of IgG. Therefore, colostrum for colostrum bank should be collected at the first 2-3 post-partum milkings according to proper harvesting and handling procedures to guarantee the safety and quality of colostrum. In addition, it was recommended that colostrum should be heat-treated before frozen and stored in the bank because pasteurization at 63°C for 30 min was very effective reducing the risk of disease transmission without causing significant degradation of immunoglobulins.

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초유은행에서 수거한 초유의 병원체 오염과 면역수준의 평가

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요 약 : 송아지는 초유를 통해서만 모체항체를 받을 수 있기 때문에 신생우가 분만초기에 초유를 섭취하는 것은 질병에 대한 저항성을 갖추기 위해 필수적이다. 따라서 분만 후 잉여 초유를 저장하였다가 초유섭취가 부족한 신생우에 공급하여 충분한 항체를 갖도록 하는 것이 일반적인 사양법이다. 본 연구에서는 국내 3곳의 지역에서 운영되는 초유은행에서 저장된 35개의 초유를 무작위로 수거하여 일반세균오염과 주요 병원체들을 검사하였고 면역글로불린의 농도와 BVDV 특이 항체를 측정하여 초유의 면역학적 품질을 평가하였다. 초유은행에서 수거된 대부분의 초유에서 중등도에서 고도의 세균오염이 관찰되었다. 또한 대부분의 초유가 좋은 품질의 초유라고 판단되는 50 g/L의 IgG 농도에 미치지 못하는 IgG를 포함하고 있었다. 따라서 초유은행 보관용 초유는 청결한 채취법에 따라 분만 후 2-3회 이하로 착유한 초유를 사용하여 안전성과 품질을 보장하여야 할 것이다. 또한 63°C에서 30분간 실시하는 저온살균이 면역글로불린의 파괴를 최소화하는 동시에 초유에 의한 질병전파를 확연히 줄여주는 것으로 관찰되었으므로 냉동보관 전에 초유의 저온살균 과정을 반드시 실시하는 것이 필요할 것으로 판단된다.

주요어 : 소 초유, 초유은행, 세균오염, 소 병원체, 면역글로불린