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Bacteriolgical Studies on Hygienic Status of the Market Bovine Liver

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Introduction

The bovine liver has been under service in the form of uncooked slice in Korean restaurant, likewise in case of slice raw fish (SASHIMI) in Japan. The liver has been frozen or refrigerated and is cut upon demand by consumers in market. Usually bovine liver is roasted on skewers or on grill but sometimes is to be eaten as it is.

There have been several reports[1,2,10] about microbial contamination of the bovine liver, in which the possibility of agonal inversion of deep tissues by enteric bacteria through mucosal surface is discussed, however reports of the hygienic study about market liver have not been found yet.

Therefore, this paper deals with the counts of indicator organisms, psychrotrophs and staphylococci as well as the common food-poisoning pathogens with a view of food sanitation.

Materials and Methods

Sampling: Samples of the bovine liver were collected aseptically at the four stores (A–D) and in the abattoir in Kagoshima City. In the latter case, the normal-looking portion of the liver condemned partially with abscess was sampled in addition to the healthy liver. Ten g of each sample was ground with 90 ml of sterile saline and the resulting suspension was subjected to bacterial examination.

Bacterial counts: A tenfold dilution with saline was made and 1 ml of each dilution was dispensed into petri dish. Three kinds of poured plates were prepared for different bacterial groups, respectively, namely, standard agar (Eiken Chemical Co.) for standard plate count (SPC) and psychrotrophs, desoxycholate agar (Eiken) for coliforms and Staphylococcus agar No. 110 (Eiken) for staphylococci. They were incubated for 2 days at 37°C for SPC, overnight at 37°C for coliforms, for 10 days at about 6°C for psychrotrophs and 2 days at 37°C for staphylococci. Colonies peculiar to the respective groups were counted and Log_{10} number of bacteria per 1 g of liver was caliculated.

***Staph. aureus*:** Seven of the colonies randomly selected from the above Staphylococcus agar No. 110 were examined for coagulase-activity, using the tube test with rabbit plasma.

***Salmonella*:** One ml of samples were added to 10 ml of Hajna tetrathionate broth (Eiken) and incubated for 2 days at 37°C. A loopful was streaked on deoxycholate hydrogen sulfide lactose agar (Nissui Pharmaceutical Co.). After overnight incubation at 37°C, *Salmonella*-like colonies were picked from these plates and were confirmed by biochemical and serological tests.

***Clostridium perfringens*:** One ml of samples were added to thioglycolate broth (Eiken) and incubated for 2 days at 37°C. A drop was streaked on CW agar (Eiken) and incubated for 2

days at 37°C in anaerobic jars (BBL GasPak system). *C. perfringens*-like colonies surrounded with iridescent layer were picked from these plates and confirmed with the aid of *C. perfringens* type-A antitoxin (Differentiation Strip, Nissui).

Antimicrobial resistance in *Escherichia coli*: One ml of samples were added to Enterococcus coliformatory broth (Nissui) and incubated overnight at 44.5°C in water bath. A loopful was streaked on Eosin-methylen blue agar (Nissui). After overnight incubation at 37°C, colonies with metalic luster were identified as *E. coli*. Antimicrobial resistance of isolates was determined by the agar dilution method[5]. The resistance concentrations of drugs were as follows: 25 μg/ml for aminobenzyl penicillin (ABPC), streptomycin (SM), tetracycline (TC), chloramphenicol (CP) and kanamycin (KM); 800 μg/ml for sulfadimethoxine (SDM).

Results

Bacterial counts: Numbers of SPC, coliforms, psychrotrophs and staphylococci averaged 6.46, 5.33, 6.34 and 5.75 respectively (Table 1). Although these counts ranged widely, they were confirmed to be distributed normally. The average numbers for the stores A–D are shown in Fig. 1, and the statistical analyses are summarized in Table 2. The level of significance by analysis of variance for SPC was 10%, and only the difference between the stores B and D was significant. Counts of coliforms, psychrotrophs and staphylococci were varied widely with the kinds of stores. On the whole, the counts of the stores A and B were less than those of the stores C and D.

Any of these four bacterial counts were not significantly different between the samples of the normal-looking portion of the abscessed liver and those of healthy liver. The average bacterial numbers in the abattoir are compared with those in market in Fig. 2. Counts of SPC and staphylococci were originally high in the abattoir while those of psychrotrophs increased markedly in market in spite of the low counts in abattoir.

Table 1. Frequency distribution of bacterial counts of the bovine liver at the market in Kagoshima

Class	SPC	Coliforms	Psychro.	Staph.
3.0–3.5	0	3	0	0
3.5–4.0	0	0	0	0
4.0–4.5	2	6	0	1
4.5–5.0	0	11	6	6
5.0–5.5	5	11	4	12
5.5–6.0	9	6	9	12
6.0–6.5	7	6	9	9
6.5–7.0	11	5	3	7
7.0–7.5	10	1	14	2
7.5–8.0	3	0	0	0
8.0–8.5	2	0	4	0
Minimum	4.38	3.48	4.51	4.38
Maximum	8.20	7.08	8.41	7.45
Mean	6.46	5.33	6.34	5.75
S. E.	0.13	0.13	0.15	0.10

S. E.: Standard error.

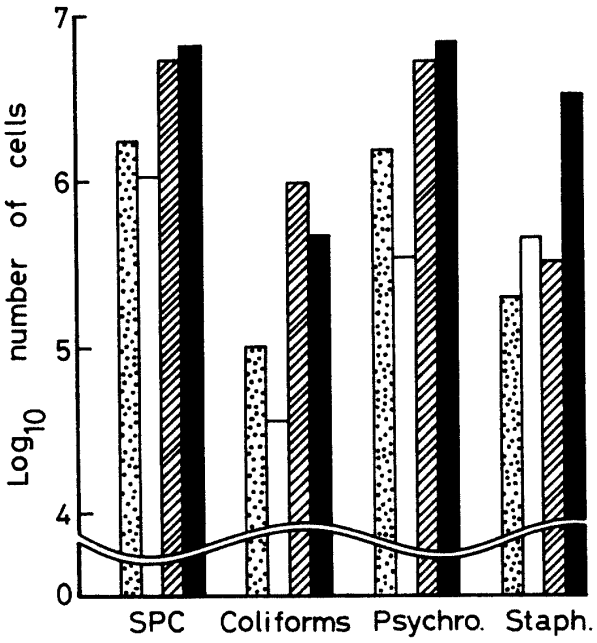

Fig. 1. Comparison of the bacterial counts of the store A(▨), B(□), C(▨), D(■).

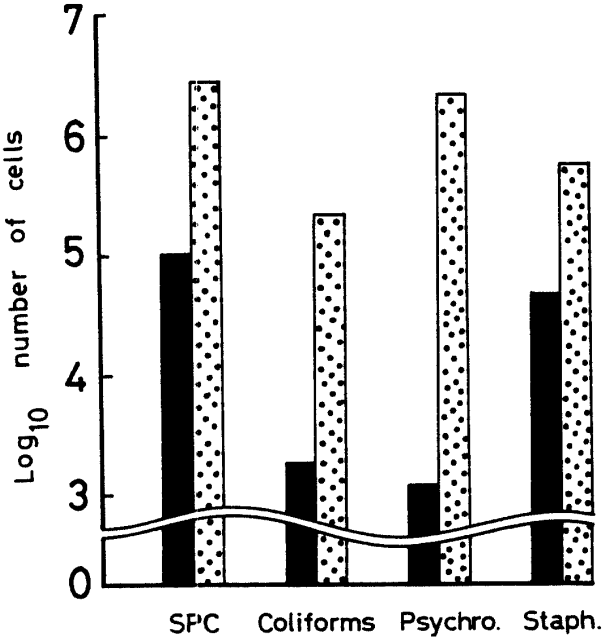

Fig. 2. Bacterial count of the bovine liver in the abattoir (■) and at the market (▨).

Table 2. Summary of statistical analyses of bacterial counts in the stores A–D

SPC (10)

	A	B	C	D
A				
B				5
C	1	1		
D	5	1		

Coliforms (1)

Psychro. (1)

	A	B	C	D
A				
B			1	1
C				
D	1	1	1	

Staph. (1)

Figures in the table show the level of significance.

Table 3. Correlation coefficient among bacterial counts

SPC			
0.35	Coliforms		
0.53	0.52	Psychro.	
0.42	0.22	0.40	Staph.

There were no large differences in correlation coefficient between the counts of any two groups (Table 3).

Food-poisoning pathogens: *Staph. aureus* was detected in 23 out of 42 samples (Table 4). The percentages of samples containing these organisms from the stores A, B, C and D were 63.6, 30.0, 60.0 and 63.6, respectively. Thirty three of 294 isolates of staphylococci (11.2%) were positive in coagulase test, and the average number of *Staph. aureus* was estimated about 4.80. In half of the samples from the abattoir, *Staph. aureus* was also detected, and 13 out of 115 isolates of staphylococci (11.3%) produced coagulase.

Table 4. Incidence of food-poisoning bacteria in the bovine liver

	Number of samples	Number of positive samples (%)
Staph. aureus	42	23 (54.8)
Salmonella	49	1 (2.0)
C. perfrigens	49	2 (4.1)

Salmonella was detected in the only one sample from the store D, and was identified as *S. anatum*. Two samples from the stores C and D contained *C. perfringens*. These samples were also contaminated with *Staph. aureus*. From 18 samples in the abattoir, *Salmonella* or *C. perfringens* were not detected.

Antimicrobial resistance: Of 54 *E. coli* isolates, 35 (64.8%) were resistant at least to one drug, and multiple resistances to 3 or more of the drugs formed 37% (Table 5). A high percentage of the isolates was resistant to TC, SM and SDM.

Characteristics of contamination: Counts of SPC, coliforms, psychrotrophs and staphylococci were normalized by the formula: (original value — mean value)/standard deviation. These normalized mean values for the stores A–D are illustrated in Fig. 3. The size of the quadrangle shows

Table 5. Drug resistance patterns in *E. coli* isolated from the bovine livers procured at market

Drug	ABPC	SM	TC	CP	KM	SDM	Number of strains	(%)
	+	+	+	+		+	2	
5	+	+	+		+	+	1	3 (5.6)
	+	+	+	+			2	
	+	+	+			+	2	
4		+	+	+		+	3	8 (14.8)
		+	+		+	+	1	
	+		+		+		1	
	+		+			+	2	
3		+	+		+		1	9 (16.7)
		+	+			+	4	
			+	+		+	1	
		+	+				2	
		+			+		1	
		+				+	1	
2			+	+			2	8 (14.8)
				+		+	1	
					+	+	1	
	+						5	
1			+				1	7 (13.0)
				+			1	
0								19 (35.0)
Number of strains (%)	16 (29.6)	20 (37.0)	25 (46.3)	12 (22.2)	6 (11.1)	19 (35.2)		54 (100.0)

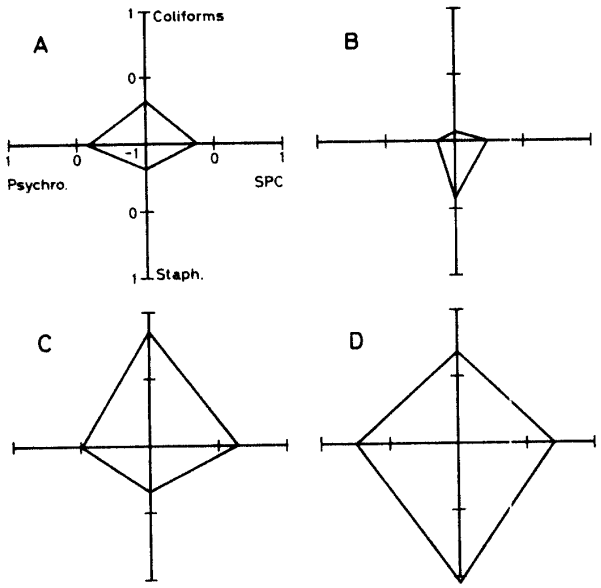

Fig. 3. Pattern of the bacterial contamination in terms of normalized values of the stores A–D.

Table 6. Principal component analysis of bacterial counts

Principal component	Element of eigenvector				Eigen value	Ratio of contribution
	SPC	Coliforms	Psychro.	Staph.		
1st	0.52	0.47	0.56	0.44	2.24	0.56
2nd	0.20	−0.66	−0.18	0.70	0.80	0.20
3rd	−0.74	0.38	−0.07	0.56	0.55	0.14
4th	0.38	0.45	−0.80	0.10	0.41	0.10

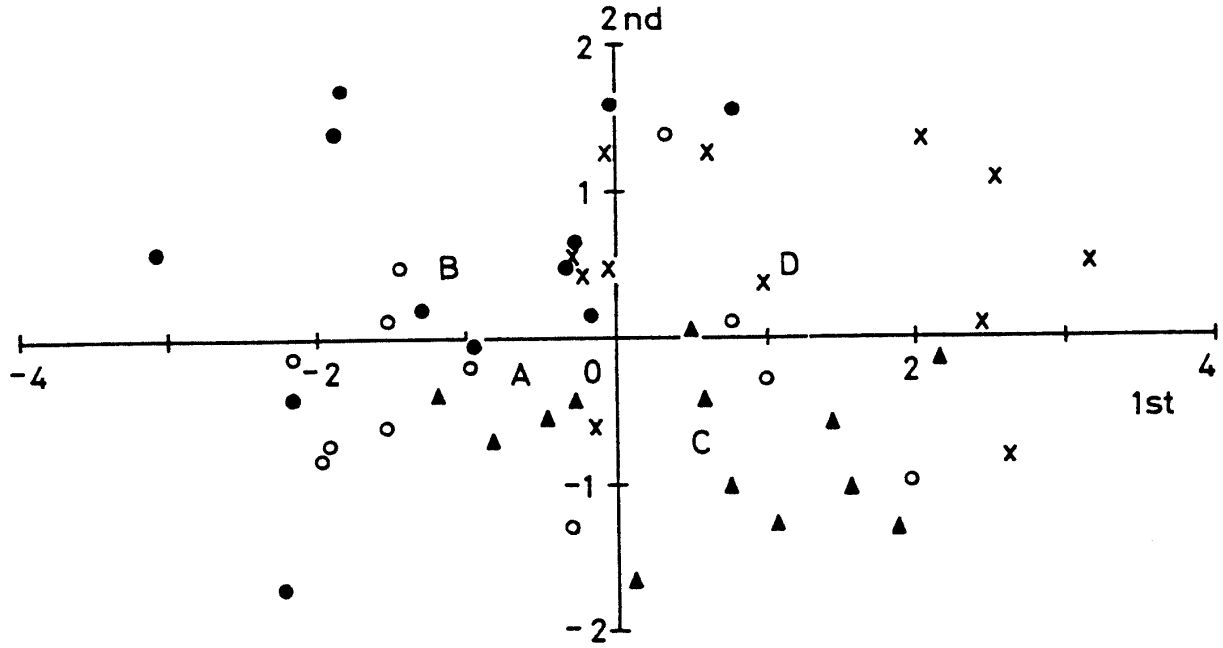

Fig. 4. Dispersion of the principal component scores. The alphabet A–D in the figure shows the mean value of the stores A(○), B(●), C(▲), D(×).

Table 7. Difference of the principal component scores of the stores A–D

1 st (1) \ 2 nd (1)	A	B	C	D
A			5	1
B	5		1	1
C		1		
D	5		1	

the degree of contamination, and so samples of the stores C and D appear to have been heavily contaminated, compared to those of the stores A and B. The shape of the quadrangle shows the property of contamination.

Results of principal component analyses are presented in Table 6. Sum of 1st and 2nd principal components held 76% of contribution. Judging by the sign of the element of eigenvector, 1st and 2nd principal components indicated the degree and the property of contamination, respctively, likewise in case of the above graphic method. The mean of the 1st principal component scores of the stores C and D were significantly larger than those of the stores A and B (Fig. 4, Table 7). The 2nd principal component scores were different significantly between stores A, C and those B, D.

Discussion

The liver from healthy animals was considered to be sterile before slaughtering, although intrinsic bacteria originated from the gut might have reached the tissue by an internal route before or after death[1,2,3,10]. In this study, bacterial counts of the healthy liver were not different from those of the normal-looking portion of the abcessed liver. Excepting some pathogenic bacteria, contamination appears to have taken place extrinsically after dressing.

Microbiological criteria for raw meat established by the individual state in America range 5–7.2 for SPC and 2–4 for coliforms[7,8]. Of the liver examined, SPC both in market and in the abattoir satisfied these criteria, but the count of coliforms in market was over the standard values. It was not comfortable, whereas Tompkin[14] asserted that the level of coliforms of raw meat after they had left the slaughter house was of questionable value. Count of SPC was not so much different from that of the bovine meat in the Japanese market[6].

The count of psychrotrophs in market was extremely higher than that in the abattoir. The ratio of psychrotrophs to SPC was 76% in market and 1.1% in the abattoir. Newton et al.[11] reported that the ratio in beef after dressing was 5.09%. It was estimated that psychrotrophs were multiplied about 11 times within the interval between the abattoir and the market.

The count of staphylococci was originally high in the abattoir and the percentage of coagulase-positive isolates was over 10%, which was almost equal to that in market. The ratio of staphylococci to SPC was 19.5 in the abattoir and 43.7 in the market. As *Staph. aureus* was detected in more than 50% of the market liver and its count was estimated to be quite high, the contamination by this organism should be taken seriously. *Salmonella anatum* and *C. perfringens* were also detected. Since these food-poisoning pathogens were distributed widely in the bovine[1,2,10,13] and many cases of food poisoning by them have been reported[14], the liver might not be proper as raw food.

Although isolation frequency of drug-resistant *E. coli* from bovine liver was lower than that from pork[12] or those from the feces of bovine or swine[14], there was a common inclination that percentages of isolates resistant to TC, SM and SDM were high. It might be a serious problem in transmission of drug-resistance that these resistant organisms of *E. coli* should have passed into human gut by eating the liver raw.

None of the two counts of these four organisms was correlate closely. Although SPC and staphylococci are both mesophilic bacteria, the correlation coefficient between the two was only 0.42, which was smaller than that between SPC and psychrotrophs. Therefore, it seemed quite reasonable for us to assume that these four organisms should have indicated different profiles of bacterial contamination. Miskimin et al.[9] reported that correlation coefficients between SPC and coliforms and between SPC and *Staph. aureus* were 0.49 and 0.34, respectively. They also emphasized that none of the indicator organisms including SPC, coliforms and *E. coli* was not suitable as a screening agent to predict the contamination by specific food-poisoning pathogens.

In order to evaluate compositely the microbiological quality, principal component analyses of these four bacterial counts were performed. The 1st and 2nd principal components held 76% of information in this survey. The principal component scores of the four stores revealed numerically the degree and the property of the microbial contamination in those stores, which were compatible with the image by the graphic method. These numerical composite data might be essential in evaluating or guiding the handling of meats. By performing further survey of the factors in handling the liver, more accurate meanings of the principal components should be clarified.

Summary

The microbiological quality and the safety of the bovine liver were investigated. Counts of SPC, coliforms, psychrotrophs and staphylococci of the market liver were 6.46, 5.33, 6.34 and 5.75, respectively. More than 50% of samples contained *Staph. aureus* and its count was estimated about 4.80. *Salmonella anatum* and *C. perfringens* were also detected. These results indicated that the bovine liver was microbiologically contaminated as heavily as the raw meat and that the habit of eating raw liver was undesirable.

By the principal component analyses of the above four bacterial counts, the degree and the property of bacterial contamination in market were numerically summarized which might be useful to evaluate and guide the propriety of handling the meat.

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