

Bacterial Count from Bovine Carcasses as an Indicator of Hygiene at Slaughtering Places: A Proposal for Sampling

JORGE A. LASTA,* RICARDO RODRÍGUEZ, MARTA ZANELLI¹, AND CARLOS A. MARGARÍA

Instituto de Tecnología de Carnes, CICV-INTA C.C. 77, 1708 Morón, Buenos Aires, Argentina

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ABSTRACT

A sampling technique by which the whole carcass is rubbed with a polyurethane sponge was used to study bacterial status on 523 beef carcasses at six different slaughterhouses over four different years. Although some abattoirs were differentiated based upon the psychrotroph counts from their carcasses, effects on counts of visits and season of sample taking, as well as interaction year x abattoir found at the other plants were large enough to mask the abattoir effect. Mesophile counts were not consistent enough to discriminate abattoirs, while, Enterobacteria, total and fecal coliforms, and *Staphylococcus aureus* coagulase-positive organisms showed very low counts and did not set apart differences. A guideline to monitor beef carcass hygiene and indirectly the hygiene of the slaughtering practices through the psychrotroph counts is proposed. A two-kinds sampling plan is suggested with "right-incorrect" as levels of hygiene. A sample unit (n) of 10, an acceptance number of contaminated carcasses (c) of 3, and a count limit (m) of 10^3 CFU/cm² are proposed. Under this guideline, a lot of carcasses will be deemed as hygiene lacking when 4 or more, out of 10 carcasses, yield counts of 10^3 CFU/cm² or higher.

Customarily, "hygienic slaughter" has been monitored through visual inspection. However, association of meat with foodborne diseases and the need of extending meat shelf life have suggested monitoring carcass microbial condition (18). Several sampling techniques such as the use of excising methods (4,10), the utilization of swabs or sponges (10,15), as well as rinsing (6,10) and self-adherence procedures (3,10), have been proposed to estimate bacterial counts on bovine carcass surfaces. Swabs counts are significantly lower than counts obtained from excision sampling procedures. However, excising methods are unacceptable for examining an entire carcass surface.

Sponges made of polyurethane have been used to detect bacteria on food plant equipment surfaces, walls, work benches, and on red meat carcasses (13). Some of the advantages of using polyurethane sponges in surface sam-

pling are ability to sample large areas, detection of low level of contamination, and no need of using glass containers. In addition, it makes sample collection easier and it has a low cost of operation (15). Furthermore, it has been reported that polyurethane sponges did not exert antibacterial activity when checked against several strains of pathogens and spoilage bacteria (13).

There is a lack of information, however, on the significance of counts obtained from abattoirs with different hygiene condition when applying the same sampling technique (9,11,12,16). Although microbial average counts have been reported to be generally lower in abattoirs with high levels of hygiene, variability of counts has been so high that such means were not significantly different unless many samples were taken. Differences in counts of up to 2 log units have been found, when the same sampling sites from carcasses at the same abattoir were compared. Even greater differences were found when different sampling sites from the same carcass were analyzed (17).

It is generally accepted that the use of Good Manufacturing Practices (GMP's) at the slaughterhouse will correlate with low carcass bacterial counts. However, even under GMP's some bacterial carcass contamination inherent to slaughtering can be expected. On the other hand, contamination is primarily uneven because of accidental contact with contaminated materials. Thus, sampling small areas will be inappropriate to determine differences between abattoirs (12). Other factors, such as visit to the abattoir for sample-taking and season when samples are taken, might also affect carcass counts.

The aims of this paper were to assess the influence of different variables that might influence the bacterial load on beef carcasses, and characterize the slaughter hygiene by means of a technique in which the whole carcass is sampled with a polyurethane sponge.

MATERIALS AND METHODS

Abattoirs (plants)

Six abattoirs were chosen, three (abattoirs: A, B, and C) were regarded as having "very good" and the other three (abattoirs: D, E, and F) as having "good" conditions of hygiene. They were

* Corresponding author.

¹ Departamento de Estadística, INTA, Cerviño 3101, 1425 Buenos Aires, Argentina.

differentiated by using an evaluation procedure developed by the Argentine Federal Meat Inspection Service, which takes into account slaughtering practices and facility characteristics (1). This evaluation protocol assigns an index to the abattoir infrastructure and to each one of the operations carried out at the slaughterhouse which will characterize the plant hygiene condition. The abattoirs, evaluated prior to sampling, were scored as "very good," and "good," respectively.

Visit to the abattoir

Abattoirs were analyzed in twos each week. Samples were taken on 2 d of a week (one per abattoir), from Tuesday to Thursday, during a 4 week-run corresponding to a typical month of summer, winter, and fall; 523 beef carcasses were sampled within the six abattoirs over four different years. See Table 1 for sampling schedule.

Determination of carcass areas

Carcass surface areas were calculated by measuring actual beef carcasses. Carcasses were diagrammatically divided into two figures, one triangle (ABE) and one trapezoid (BCDE) (Fig. 1). The sides of these figures were calculated by measuring the length of the segments AB, BE, AE, BC, CD, and DE shown on Fig. 1. Segments BC and DE correspond to dorsal and ventral lengths of

thoracic and abdominal walls, respectively, whereas BE represents the horizontal distance from the tail vertebrae to the ventral and distal part of the abdominal wall. Finally, segment CD is the length of the horizontal that goes from the first sternbrae of the breast bone to the first feather bone.

Beef carcass areas were estimated by adding up the areas of the triangle and the trapezoid. Relationships between the calculated carcass areas and carcass weights were calculated by correlation coefficient (r), for each one of the cattle market classes considered (steer and heifer) (2).

Considered factors

Abattoir, visit, day of the week, season, and year in which the visit was carried out, as well as cattle market class and carcass grade (fat thickness) (2) were considered to be factors that might influence carcass bacterial load.

Carcass sampling

Beef carcasses were sampled at the end of the slaughter line and immediately before entering the chilling room. A sterile plastic bag (30 x 40 cm and 50 μ m thick) was inverted in an aseptic manner on the hand of the technician that took the sample, in that way the internal bag side appeared to be external. With the operator's hand protected in such a way, a sterilized polyurethane

TABLE 1. Sampling schedule showing visits to abattoirs "very good" A, B, and C and "good" D, E, and F, respectively, counts performed, and data organization.

Data subset	Year	Season	Visits ^a to abattoirs "very good"				Visits to abattoirs "good"				Counts
			1	2	3	4	1	2	3	4	
I	1	SUM WIN	A				D				Psychrotrophs
			10	10	9	8	10	10	10	7	
II	2	SUM WIN	B				E				Psychrotrophs Mesophiles
			10	9	9	10	10	10	9	9	
III ^b	3	SUM WIN	A				D				Psychrotrophs Mesophiles Enterobacteria Total coliforms Fecal coliforms <i>S. aureus</i>
			8	10	10	10	10	8	10	10	
IV	4	AUT	C				F				Psychrotrophs Mesophiles Enterobacteria Total coliforms Fecal coliforms <i>S. aureus</i>
			10	10	10	9	10	10	9	10	
V ^c	1	SUM	A				D				Psychrotrophs
	2	WIN	B				E				Psychrotrophs
	3	SUM WIN	A				D				Psychrotrophs

^a At each visit the number of beef carcasses sampled is shown.

^b Psychrotroph counts were analyzed with data subset I.

^c Data subset V = data subset I + data subset II + data subset III.

RESULTS

Carcass areas

The relationship between carcass area and carcass weight of different cattle market classes showed an r value of >0.911 ($P < 0.05$). Since a scale for each market class was set at the beginning of the study, it was possible to estimate the carcass areas sampled (Table 3).

TABLE 3. Relationship between carcass weight and carcass area for steers and heifers ($r > 0.911$).

Cattle market class	Carcass weight ^a (kg)	Carcass area (cm ²)
Steers	100-104	16,805
	105-114	17,226
	115-124	17,307
	125-134	17,623
	135-144	18,475
	145-154	18,750
	155-164	20,817
	165-174	23,160
Heifers	70-74	13,351
	75-79	15,462
	80-84	15,832
	85-89	15,932
	90-94	16,446
	95-99	17,116

^a Ten carcasses were considered for each category of weight.

Data subset I

The overall mean (log CFU/cm²) for the total viable count of psychrotroph was 2.14 within this data subset, with a variance of 1.16. Data subset I was analyzed with psychrotroph counts from data subset III because they were derived from the same slaughterhouses [(A) and (D), respectively]. Lower psychrotroph counts were found at abattoir scored as "very good" when compared with counts from "good" abattoir (Table 5). However, the effects of season, visit and year x abattoir hid the abattoir effect (Table 4). The residual scatter plot for counts from this data subset is shown on Fig. 2. Each residual refers to an observation from "very good" (A) or "good" (D) abattoir. Three fields can be identified thereon: the one on the left where "very good" prevails (with low number of bacteria), the one on the right with a widespread of "good" and higher prediction values, and the one in the middle where both residuals are mixed.

Data subset II

The counts of psychrotroph (log CFU/cm²) yielded a mean of 2.19 with a variance of 1.23, whereas the mesophiles mean (log CFU/cm²) was 1.92 with a variance of 2.09. The analysis of variance for psychrotroph and mesophile is shown on Table 4. There were no differences between counts in different seasons and for the abattoir x season interaction. However, when analyzing the effect of visit within the same season and abattoir, and the effect of abattoirs on counts, these were significant. Such condition allowed differentiation of abattoirs based on psychrotroph

TABLE 4. Analysis of sources of variation (effects) when comparing mean counts (log CFU/cm²) of psychrotrophs (PSYCH) and mesophiles (MESOPH) on beef carcasses from different abattoirs. A significance level of $P < 0.05$ (*) and $P > 0.05$ (NS) is used.

Effect	Data subset					
	I ^a	II ^b		IV ^c		V ^d
	PSYCH	PSYCH	MESOPH	PSYCH	MESOPH	PSYCH
Abattoir	NC	*	*	*	NS	NC
Year	NC	-	-	-	-	NC
Season (year)	*	NS	NS	-	-	*
Visit (abattoir, season, year)	*	*	*	*	NS	NS
Year x abattoir	*	-	-	-	-	*
Abattoir x season	NS	NS	NS	-	-	NS

^a Data subset I: abattoirs "very good" (A) and "good" (D).

^b Data subset II: abattoirs "very good" (B) and "good" (E).

^c Data subset IV: abattoirs "very good" (C) and "good" (F).

^d Data subset V: data subset I + data subset II + data subset III. (NC) Due to interactions main effects are not compared. (-) Effect not included in the corresponding statistical model.

TABLE 5. Least squares means for psychrotroph counts^a when analyzing the effect of visit (abattoir, season, year) from data subset I [abattoirs "very good" (A) and "good" (D)].

Year	Season	Abattoir							
		A				D			
		Visit							
		1	2	3	4	1	2	3	4
1	Winter	1.63	2.02	1.79	2.11	2.77	2.16	2.81	2.23
	Summer	1.51	1.33	1.20	1.53	1.65	2.79	1.95	2.09
3	Winter	1.66	1.71	1.47	1.75	2.48	2.35	2.83	2.99
	Summer	1.78	1.44	1.67	2.33	2.40	3.28	3.52	3.54

^a Psychrotroph counts on beef carcasses are log CFU/cm².

and mesophile counts (Table 6). Finally, Fig. 3 shows the residuals from the psychrotroph counts compared to their prediction values. Except for a few high prediction values from the abattoir "very good" (B), residuals from both abattoirs seem to be placed separately in two definite fields.

Data subset IV

The overall mean of psychrotroph counts (log CFU/cm²) resulted in 2.70 and the variance 2.75, while the

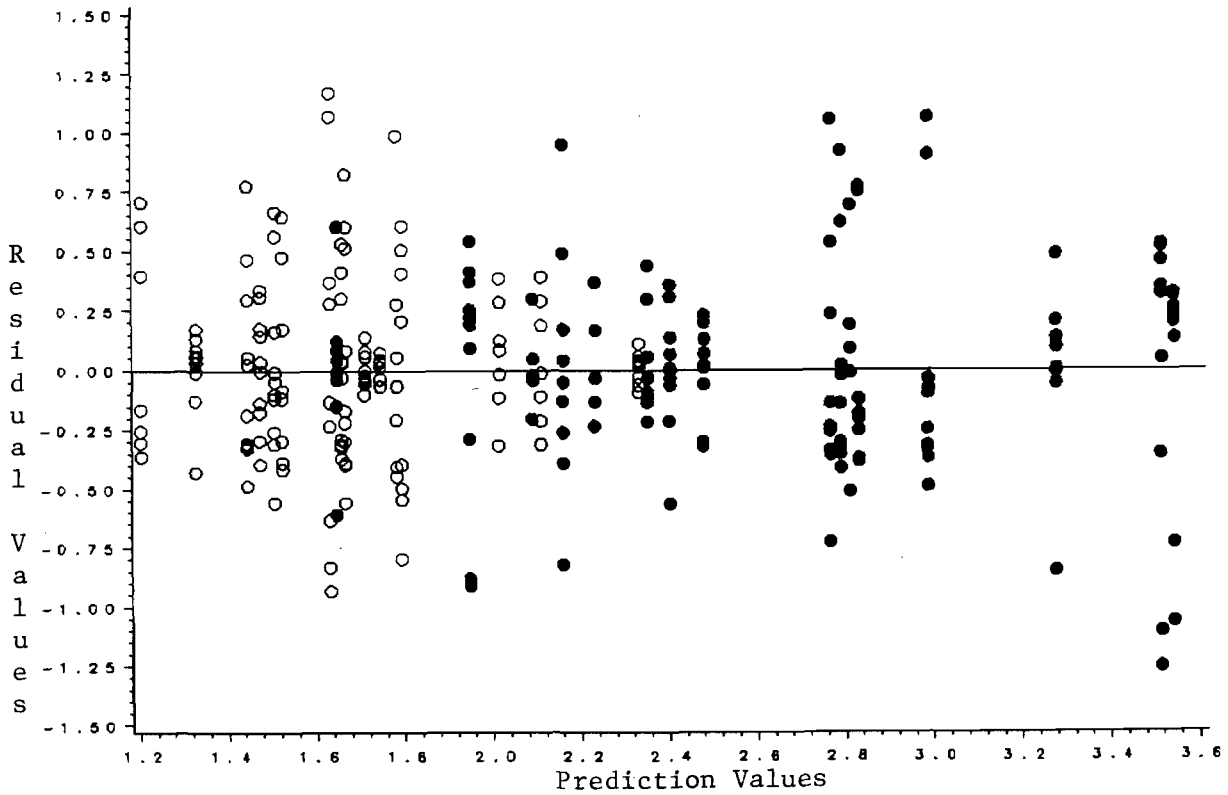


Figure 2. Residual plot from psychrotrophs count^a for data subset I^b (79 observations are hidden).

^a Psychrotroph counts on beef carcasses are log CFU/cm².

^b ○ observations from abattoir “very good” (A).

● observations from abattoir “good” (D).

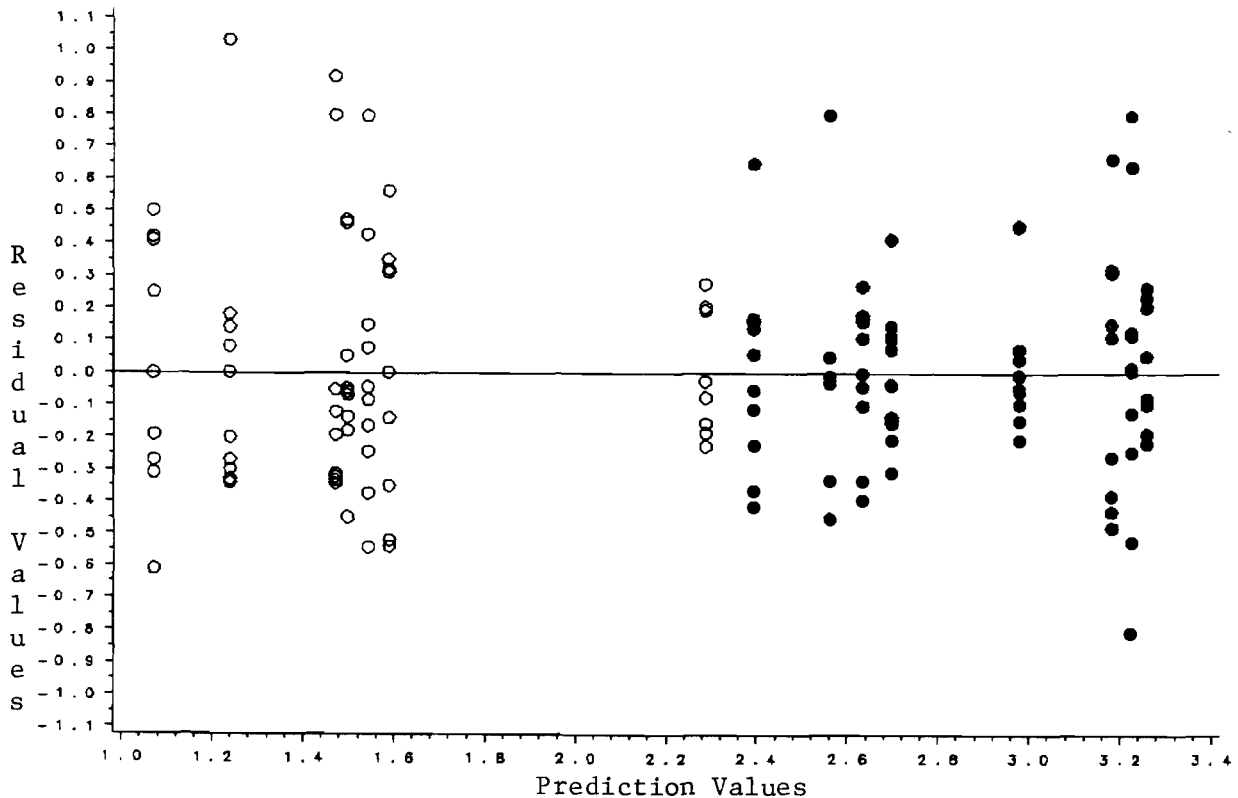


Figure 3. Residual plot from psychrotroph counts^a for data subset II^b (28 observations are hidden).

^a Psychrotroph counts on beef carcasses are log CFU/cm².

^b ○ observations from abattoir “very good” (B).

● observations from abattoir “good” (E).

TABLE 6. Mean bacterial counts (log CFU/cm²) on beef carcasses from abattoirs scored as "very good" (VG) and "good" (G). Calculated F values are shown.

Data subset	Bacterial count	Abattoirs		Statistic F
		VG	G	
II ^a	Psychrotrophs	1.53	2.86	54.27*
	Mesophiles	1.33	2.53	26.29*
IV ^b	Psychrotrophs	2.21	3.20	7.05*
	Mesophiles	1.67	2.71	5.71 NS

^a Abattoirs "very good" (B) and "good" (E).

^b Abattoirs "very good" (C) and "good" (F).

(*) P<0.05.

(NS) P>0.05.

mesophile mean counts (log/cm²) was 2.19 with a variance of 3.73. Results within this data subset are shown on Tables 4 and 6. A high variability between visits emerged in mesophile count; consequently, counts from different abattoirs showed no differences. On the other hand, psychrotroph counts were different (P<0.05).

Data subset V (I+II+III)

The psychrotroph counts (log CFU/cm²) yielded a mean of 2.16 with a variance of 1.19. Analysis of variance is shown on Table 4 and the least square means for counts when considering abattoirs and years are shown on Table 7.

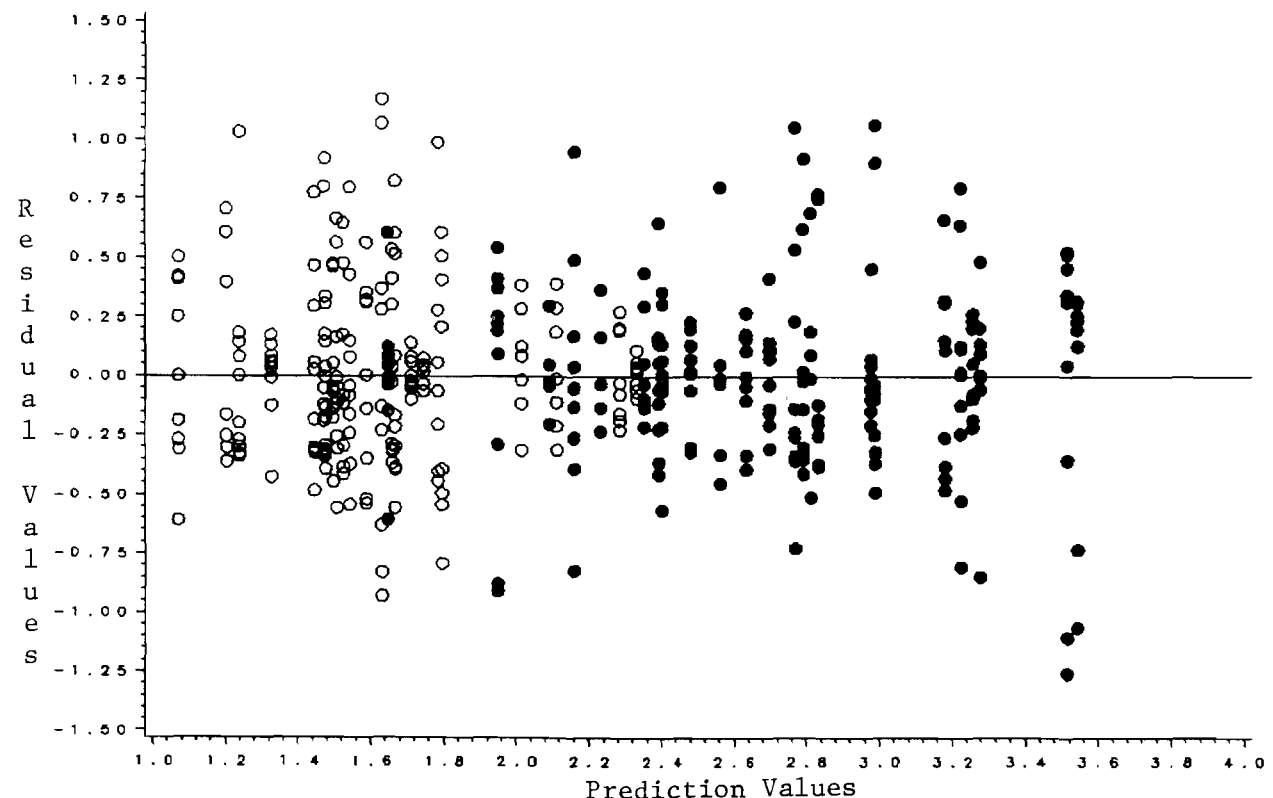


Figure 4. Residual plot from psychrotroph counts^a for data subset V^b (126 observations are hidden).

^a Psychrotroph counts on beef carcasses are log CFU/cm².

^b ○ observations from abattoirs "very good" (A) and (B).

● observations from abattoirs "good" (D) and (E).

The significance of the effect year x abattoir interaction reveals a different result of the abattoirs for the different years. Nonetheless, the psychrotrophs count from carcasses sampled at "very good" abattoirs tended to be lower than those from carcasses sampled at "good" abattoirs. An acceptable residual plot, with observations at each end of the base line corresponding to counts from "very good" and "good" abattoirs, respectively, and a medium field with values from 1.6 to 2.4, where observations are mixed, is shown on Fig. 4.

TABLE 7. Least squares means for psychrotroph counts^a when analyzing year and type of abattoir from data subset V {abattoirs "very good" [VG] (A) and (B) + abattoirs "good" [G] (D) and (E)}.

Year	Type of abattoir	
	VG	G
1	1.64	2.31
2	1.73	2.92
3	1.53	2.86

^a Psychrotroph counts on beef carcasses are log CFU/cm².

Other counts

Enterobacteria, total coliforms, and fecal coliforms yielded values lower than 1 CFU/cm² on carcasses from abattoirs scored as "very good" and in 68% of samples from "good" abattoirs. Counts of *S. aureus* coagulase positive were lower than 1 CFU/cm² at both groups of abattoirs.

Other considered factors

Cattle market class, carcass grade (fat thickness), and day of the week in which sample taking was carried out had no effect ($P>0.05$) on carcass count.

Probability of acceptance (P) of a beef carcass lot having different hygiene condition.

Calculated P values based on the proportion of carcasses contaminated with psychrotrophs are shown on Table 8. Probabilities vary according to the psychrotroph counts limit that is considered. P was higher at "very good" plants since the proportion of contaminated carcasses was lower at "very good" rather than at "good" abattoirs. P is increased as the number of contaminated carcasses is increased because it is a cumulative probability.

TABLE 8. Probabilities of acceptance (P) of a beef carcass lot^a having different hygiene condition considering a count limit of total viable psychrotrophs to be applied at "very good" (VG) and "good" (G) abattoirs.

c ^b	Type of abattoir	P			
		m ^c			
		10 ¹	10 ²	10 ³	10 ⁴
0	VG	0.00022	0.15490	0.91370	1.00000
1		0.00210	0.44460	0.99640	1.00000
2		0.01000	0.71470	0.99990	1.00000
3		0.03230	0.88210	0.99990	1.00000
0	G	0.00004	0.00018	0.06600	0.86030
1		0.00050	0.00170	0.24590	0.99010
2		0.00270	0.00850	0.49060	0.99950
3		0.01020	0.02810	0.71200	0.99990
0	VG + G	0.00009	0.00530	0.24540	0.92850
1		0.00100	0.03330	0.59110	0.99760
2		0.00530	0.10660	0.83370	0.99990
3		0.01840	0.23460	0.94670	1.00000

^a A lot of 10 beef carcasses is used as a sample unit (n).

^b Acceptable number of contaminated carcasses.

^c Count limit = CFU/cm².

DISCUSSION

To avoid the count variability found when sampling small carcass areas, a sampling technique which consisted of swabbing the whole beef carcass with a polyurethane sponge was developed. Sampling with sponges was carried out without interrupting the slaughter line activities, being a relatively quick procedure and without encountering the lack of liquid absorption reported (13). In our study, more than 520 beef carcasses (generally 10 per visit) were analyzed with an estimated sampling area ranging from 13,351 to 23,160 cm². It is pointed out that a sampling area of 12.3 cm² has been used in some studies to evaluate carcass contamination (11); furthermore, other reports have analyzed a very low (one or two) number of carcasses per visit (9).

Abattoirs scored as "very good" were discriminated from the "good" ones by the psychrotroph counts, while

mesophile counts were not consistent to that purpose (Table 6). On the other hand, counts of Enterobacteria, total and fecal coliforms and *S. aureus* did not set apart differences between groups of abattoirs. These results are coincident with other reports (11,17), particularly the generally low values for the last four counts. However, the values were lower in our study probably due to the "dilution effect" resulting from a larger area sampled. Psychrotroph counts were also low, even the ones from "good" abattoirs. The highest count was 3.20 log CFU/cm² (Table 6).

It is emphasized that the mean value of the counts correspondent to the 28 visits (Table 1) to "very good" abattoirs were lower than the mean value of the 28 visits to the ones scored as "good". It seems that the sum of better slaughtering practices and facility conditions yields consistently lower bacterial counts. This is clearly shown on the residual plots from psychrotroph counts with two end fields with exclusively either low ("very good") or high ("good") prediction values and a middle field where observations from both abattoirs are mixed (Fig. 2, 3, and 4). The presence of residuals from abattoirs "good" in the zone of low counts (Fig. 2 and 4) would suggest that these abattoirs follow GMP's. Moreover, differences between "good" and "very good" slaughterhouses were slight.

The influence of the seasons of the year was demonstrated here (Table 4), as well as its variability that could have disguised the difference between abattoirs. However, it seems that the season effect might not be consistent because it was not found on data subset II (Table 4). This is somewhat in agreement with other reports (9,11), where trends in mesophile and psychrotroph counts from carcasses sampled at different seasons were inconsistent and no significant differences were found. Season and visit effects can be so important that these should be carefully considered to avoid mutual masking. If only one visit is done per season, then it will be impossible to separate both effects.

When compared within the same abattoir, visits resulted in the most important source of variation (18). In this study, as visits were made on alternate days of the week and avoiding coincidence of the first or last day with the same abattoir, it was possible to establish clearly the differences between season and year. The year can influence counts on carcasses (9); in our study, it is not possible to discuss the effect of year on carcass bacterial load from data subsets I and V since interaction was present (Table 4). Furthermore, the year x abattoir interaction seems to play a critical role as significant differences are looked for. However, in the particular case of data subset I it might be significant because of a quantitative difference of count trend from abattoirs A and D in the two years.

In relation with carcass fatness, lamb carcasses having 0.36 cm or less of fat thickness, chilled for 4 and 7 d, yielded higher psychrotrophs counts than fatter carcasses (20). In our research, no differences were found either on counts from beef carcasses with different fat thickness (carcass grade) or on counts from steers and heifers.

Taking into account the previous discussion and the count values, it is feasible to develop a microbiological guideline and a sampling plan for monitoring hygiene of

the slaughtering process by means of the psychrotroph counts from beef carcasses. This guideline should be a tool for personnel with responsibilities on quality and production control.

A sampling plan of two kinds (7) is suggested. The microorganisms considered (psychrotrophs) do not represent a direct risk for health, but they would allow us to indicate either levels of hygiene or their influence on the product shelf life. Under the suggested sampling plan, the expression "acceptance-refusal" usually applied on two-kind plans (7) should be substituted for the one: "right-incorrect" level of hygiene. The plan will take into account whatever the industry is able to produce in accordance with the application of GMP's, considering that under the current slaughtering practices, some sort of bacterial contamination on carcasses can be expected.

The value $n=10$ (sample unit) is proposed because this number of carcasses can be analyzed without excessive expenses. The values for m (count limit) and c (acceptable number of contaminated carcasses) are also feasible of being obtained under GMP's. These values will depend on considering separately the "very good" from the "good" abattoir or both plants together. Thus, the probability of acceptance of a beef carcass lot having 0, 1, 2, or 3 carcasses contaminated with a definite bacterial level can be determined (Table 8). From this table, a "fair" beef carcass hygiene condition can be defined as the one having an acceptable bacterial load and consequently an adequated hygiene level.

As both hygiene level of plants are jointly considered, $n=10$, $c=3$, and $m=10^3$ CFU/cm² are proposed. This count or a lower one will likely be obtained through the current slaughtering practices, there being no microbiological risk in case of such a low microbial population increase due to postslaughtering operations (fabrication and merchandising). A lot of beef carcasses will be deemed as hygiene lacking when 4 or more, out of 10 carcasses, yield counts of 10^3 CFU/cm² or higher. Under this guideline it is possible to estimate probability levels (P) of having carcasses with "fair" conditions of hygiene (Table 8). If the "very good" and "good" plants are considered together, P will be equal to 0.94; if the "very good" is taken alone, P will be 0.99, whereas P will result in 0.71 if only the "good" abattoir is taken into account. It ought to be emphasized that this proposal should be regarded as a guide for the hygiene status on beef carcasses and indirectly of the slaughtering practices, serving as a piece of advice for decision making.

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