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## Control of the bacteriological condition of calf brain. I. Impact of improving hygiene

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Sixty calves of the Dutch Friesian (FH) breed were stunned mechanically. Without previously having been stunned, another 30 calves were stuck according to the Jewish rite. Upon opening of the skulls (1–2 h post mortem) brains of mechanically stunned calves were collected either conventionally ( $n = 30$ ) or 'hygienically' ( $n = 30$ ), i.e. using a fresh pair of surgical gloves during each removal to avoid cross contamination. For ritually slaughtered animals only the hygienic procedure was followed. Samples of 10 g were excised from undamaged hemispheres and in the mechanically stunned treatment group also from the site of impact of the captive bolt. After storage in polystyrene trays at  $3 \pm 1^\circ\text{C}$  for 7 days sampling was repeated. Bacteriological examination included the assessment of aerobic colony counts at  $30^\circ\text{C}$  for 3 days (ACC-30) and  $4^\circ\text{C}$  for 14 days (ACC-4) and Enterobacteriaceae colony counts at  $37^\circ\text{C}$  for 20 h (ECC). In conventionally collected samples the ACC-30 and ACC-4 were 3.8 and 3.0  $\log_{10}$  cfu  $\text{g}^{-1}$  at day 1 and 6.2 and 6.4  $\log_{10}$  cfu  $\text{g}^{-1}$  at day 8. With hygienic collection these counts were reduced by approximately 1 log unit. Whilst by conventional practice the ECCs, at day 1 and 8 were 2.6 and 4.8  $\log_{10}$  cfu  $\text{g}^{-1}$ , these counts were 1.8 and 2.6  $\log_{10}$  cfu  $\text{g}^{-1}$  for hygienic practice. In samples excised from the site of impact of the captive bolt the hygienic procedure had similar, though less marked effects. On day 1 brains from ritually slaughtered animals had a bacteriological contamination similar to that found in the hemispheres of mechanically stunned calves. However, whilst at day 8 their mean ECCs were 3.4 and 3.5  $\log_{10}$  cfu  $\text{g}^{-1}$  the percentages of plates 'positive' for Enterobacteriaceae were only 10% in the ritually vs. 53% in the mechanically stunned group. The Enterobacteriaceae in this case were composed of psychrotrophic non-pathogenic genera of environmental origin. *Salmonella* was not isolated from any sample.

Key words: Calf brain, bacteriology; Hygiene, improved

### Introduction

The bacteriological condition of edible slaughter by-products and the manner in which this is affected by various handling procedures, packaging and storage conditions has been studied recently (Patterson and Gibbs, 1979; Bijker, 1981; Berry et al., 1981; Hanna et al., 1982a, b; Oblinger et al., 1982; Rothenberg et al., 1982; Smith et al., 1983; Woolthuis et al., 1984). The by-products studied included livers, tongues, udders, kidneys, skirts and tails.

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Another by-product, viz. calf brain, has received very little attention, though it is considered a delicacy in West European countries (Fisher, 1969), usually as a fried dish (Anon., 1981). It is also used as a basic component in sausages and in soups (von Ostertag and Schönberg, 1955; Linke, 1959). Calf brain contains approximately 9% fat, 10% protein, including all essential amino acids, and it is relatively rich in B vitamins and vitamin C, compared to lean veal (Souci et al., 1969). It has been recommended as being particularly suitable for diabetes mellitus patients (Weitzel cited in von Ostertag and Schönberg, 1955).

Due to its particular tissue structure (i.e. a parenchymatous organ with very little mesenchymal interstitium) the conditions for bacterial colonization of brain are optimal. Its use in culture media for fastidious microorganisms substantiates this (Rosenow, 1919). Important factors determining the keeping quality of calf brain are (i) the extent of initial contamination as affected by manipulations and (ii) storage conditions from the moment of removal from the skull. The initial contamination is determined to a great extent by the stunning procedure. In essence three stunning methods are available for veal calves, viz. concussive, electrical, and captive bolt stunning. The latter method is generally used since it is considered the most practical one under the present conditions (Lambooy and Spanjaard, 1981). However, the captive bolt damages part of the brain, usually the cerebellum, forcing skin and bone fragments into the inner parts. Thus, brain tissue is inoculated with potentially hazardous microflora. Manual fixation of the head, splitting of the skull and removal of the brain imply other ways of contamination.

The purpose of the present study was to investigate whether paying particular attention to hygienic measures during the removal of the brain from the skull would significantly improve the bacteriological condition of brains originating from calves stunned by means of a captive bolt. Furthermore it seemed worthwhile investigating whether alternative stunning procedures not involving mechanical damaging of brain tissue might produce calf brain of superior bacteriological quality. To this end a series of experiments was conducted in which 'kosher' slaughtering (i.e. slaughtering according to the Jewish rite implying sticking without previous stunning) was used as a model for such alternatives.

## **Materials and Methods**

### *Samples*

A total of 60 calves of the Dutch Friesian (FH) breed was stunned mechanically by means of a captive bolt. Without previously having been stunned another 30 calves were stuck according to the Jewish rite. Immediately after bleeding i.e. approximately 5 min post mortem carcasses were decapitated. Approximately at 1 h post mortem (for ritually slaughtered animals at 2 h) the heads were skinned and hot boned and the skulls were opened. From heads of mechanically stunned animals the brain was removed either 'conventionally' (30 samples) or 'hygienically' (30 samples), whereas with ritually slaughtered calves only the latter procedure was followed.

'Conventional' practice in the plant implied manual fixation of the head, splitting of the occipital bone by means of an axe and removal of the brain; all these manipulations were carried out by the same person without interim cleaning of the axe. 'Hygienic' practice relied on the same manipulations with exception of the removal of the brain from the skull, which was carried out by a second person wearing a fresh pair of surgical gloves during each removal in order to avoid cross contamination. Upon removal from the skull all brains were placed in polystyrene trays and stored for 7 days at  $3 \pm 1^\circ\text{C}$  in crates which were overwrapped with aluminium film to reduce the extent of desiccation. This procedure conforms to the storage conditions in practice.

### *Bacteriological examination*

Samples for bacteriological examination were taken immediately after collection and in addition after 7 days of storage at  $3 \pm 1^\circ\text{C}$ . Using sterile surgical blades, cone-shaped samples with a base diameter of approximately 2 cm were excised from one of the hemispheres, and, in the case of brain originating from mechanically stunned animals, also from the site of impact of the captive bolt, usually the cerebellum. After maceration of 10 g brain tissue in a Stomacher (Gerats and Snijders, 1978) and resuscitation for  $1\frac{3}{4}$ –2 h at ambient temperatures in 90 ml buffered peptone water (van Leusden et al., 1982) numbers of colony forming units (cfu) of the following microorganisms were assessed:

(1) Aerobic organisms; in poured plates of tryptone glucose beef extract agar (Difco 0002); incubation 3 days at  $30^\circ\text{C}$  and 14 days at  $4^\circ\text{C}$ .

(2) Enterobacteriaceae; in poured plates of violet red bile glucose (VRBG) agar (Oxoid CM 485) with overlayer; incubation 20 h at  $37^\circ\text{C}$  (Mossel et al., 1962).

All samples resulting in plates appropriate for further examination [i.e. containing at least 7 typical Enterobacteriaceae colonies corresponding to  $\log_{10}$  cfu  $\text{g}^{-1} \geq 1.8$  (Mossel and Drion, 1954)] were tested for *Salmonella*. For this purpose the remaining macerate was incubated at  $37^\circ\text{C}$  for 24 h after which 1.0 ml was inoculated in 10 ml Muller-Kauffmann broth (Oxoid CM 343) and incubated at  $43^\circ\text{C}$  for another 24 h. After plating on brilliant green agar (Oxoid CM 329) and incubation for 24 h at  $37^\circ\text{C}$ , typical colonies were tested by assessing the type of growth in Kligler iron agar tubes (Difco 0086) and agglutination by polyvalent and subsequently group sera.

To study the composition of the Enterobacteriaceae flora of brain hygienically removed from ritually slaughtered calves, single colonies in VRBG were subcultured on MacConkey agar no. 3 (Oxoid CM 115) at  $37^\circ\text{C}$  and identified to genus level. The following criteria were used for that purpose: Gram stain, oxidase reaction, behaviour in a three-layer tube (allowing determination of mode of attack on glucose, motility and formation of indole and  $\text{H}_2\text{S}$ ), assimilation of citrate, dissimilation of urea and DNAase and gelatin activity. When required additional testing for  $\beta$ -galactosidase and attack on arabinose was done (Mossel et al., 1979).

*Statistical analysis of data.*

For both aerobic colony counts and Enterobacteriaceae counts, plates were considered appropriate for enumeration when at least 7 colonies were present, corresponding to  $\log_{10}$  cfu  $g^{-1} \geq 1.8$ . All plates appropriate for enumeration of Enterobacteriaceae were also tested for *Salmonella*.

To determine the significance of differences between counts, the means of the four treatment combinations were compared pairwise with Fisher's LSD method, i.e. the *t*-test was used with a pooled variance estimate. For this purpose samples with less than 7 colonies in plates inoculated with the first decimal dilution ( $\log_{10}$  cfu  $g^{-1} < 1.8$ ) were assigned counts of 1.8 log units.

## Results and Discussion

Table I presents the aerobic colony counts at 30°C and at 4°C, as well as colony counts of Enterobacteriaceae, expressed as  $\log_{10}$  cfu  $g^{-1}$  brain tissue excised from the hemispheres of mechanically stunned animals.

As a result of hygienic practices at day 1 the percentage of plates appropriate for enumeration was drastically reduced in all counts. In the case of Enterobacteriaceae this occurred to such an extent that these bacteria remained 'undetectable'. The initial total colony counts at 30°C and at 4°C were approximately 1.5 and 1.0  $\log_{10}$  units lower than with conventional practices ( $p < 0.001$ ).

After 7 days of storage all samples of the conventionally collected brains yielded plates appropriate for enumeration on all media. Hygienically collected samples, however, still showed 47% of the samples being 'negative' for Enterobacteriaceae. Salmonellae were not detected in any sample examined. This substantiates earlier studies on the bacteriological condition of veal in the same plant (Smulders and

TABLE I

The bacteriological condition of *undamaged hemispheres* of calf brain after conventional ( $n = 30$ ) and hygienic ( $n = 30$ ) collection, measured initially (day 1) and after 7 days of storage at  $3 \pm 1^\circ\text{C}$  (day 8); plates appropriate for enumeration (%), mean bacterial colony counts and standard deviations ( $\log_{10}$  cfu  $g^{-1}$ )

	Day 1		Day 8	
	Conventional	Hygienic	Conventional	Hygienic
Aerobic colony count (3 days, 30°C)	100% 3.8 ± 0.6 <sup>b,*</sup>	60% 2.3 ± 0.3 <sup>a</sup>	100% 6.2 ± 0.7 <sup>d</sup>	100% 5.1 ± 0.7 <sup>c</sup>
Aerobic colony count (14 days, 4°C)	97% 3.0 ± 0.5 <sup>b</sup>	20% 2.1 ± 0.1 <sup>a</sup>	100% 6.4 ± 0.8 <sup>d</sup>	100% 5.2 ± 0.6 <sup>c</sup>
Enterobacteriaceae colony count (20 h, 37°C)	30% 2.6 ± 0.4 <sup>b</sup>	0% < 1.8 <sup>a</sup>	100% 4.8 ± 0.6 <sup>c</sup>	53% 3.4 ± 0.8 <sup>c</sup>

\* In horizontal rows figures with different superscripts differ significantly ( $p < 0.05$ ).

Woolthuis, 1983), and is indicative of a relatively high standard of basic plant hygiene (Hobbs, 1964).

Table II presents aerobic colony counts at 30 and 4°C as well as counts of Enterobacteriaceae expressed a  $\log_{10}$  cfu  $g^{-1}$  brain tissue excised from the site of impact of the captive bolt. These results show that with respect to damaged brain areas, hygiene results in a less marked reduction of the percentage of plates appropriate for enumeration as compared with intact hemispheres: on day 1 only aerobic colony counts at 4°C and Enterobacteriaceae counts showed smaller percentages than controls did. However, all mean bacterial counts on day 1 were more than 1  $\log_{10}$  unit lower. At day 8 only Enterobacteriaceae counts showed a slightly reduced percentage of countable plates ( $p < 0.05$ ) whilst mean values for total colony counts at 30 and 4°C and Enterobacteriaceae are reduced by 0.4, 0.5 and 0.8  $\log_{10}$  units respectively ( $p < 0.025$ ). All samples positive for Enterobacteriaceae were examined for Salmonellae, but none were found to be positive. These results show that, in spite of the inevitable contamination of the brain with skin fragments in the course of the stunning process, hygienic practices may yet improve the bacteriological condition significantly.

Table III presents data on the bacteriological condition of brain originating from ritually slaughtered calves expressed as  $\log_{10}$  cfu  $g^{-1}$ . On day 1 the percentage of plates appropriate for enumeration was lower than with mechanical stunning, this being particularly evident when comparing the data with those on the damaged tissue (Table II, row 'hygienic'). All mean bacterial counts on day 1 were extremely low. For aerobic colony counts they hardly exceeded, and for Enterobacteriaceae counts were even less than their respective limits of detection, i.e.  $\log_{10} = 1.8$ . Nonetheless, these figures were not significantly lower than those applying to hemisphere samples obtained by the hygienic procedure. This finding clearly illustrates that hygienic practices will suffice to prevent dispersion of bacteria from contaminated areas such as the site of impact of the captive bolt.

TABLE II

The bacteriological condition of calf brain at the *site of impact* of the captive bolt after conventional ( $n = 30$ ) and hygienic ( $n = 30$ ) collection, measured initially (day 1) and after 7 days of storage at  $3 \pm 1^\circ\text{C}$  (day 8); plates appropriate for enumeration (%), mean bacterial colony counts and standard deviation ( $\log_{10}$  cfu  $g^{-1}$ )

	Day 1		Day 8	
	Conventional	Hygienic	Conventional	Hygienic
Aerobic colony count (3 days, 30°C)	100% 5.0 ± 0.9 <sup>b,*</sup>	100% 3.8 ± 0.8 <sup>a</sup>	100% 6.9 ± 0.7 <sup>c</sup>	100% 6.5 ± 0.8 <sup>c</sup>
Aerobic colony count (14 days, 4°C)	100% 3.8 ± 0.7 <sup>b</sup>	63% 2.6 ± 0.5 <sup>a</sup>	100% 7.0 ± 0.7 <sup>d</sup>	100% 6.5 ± 0.8 <sup>c</sup>
Enterobacteriaceae colony count (20 h, 17°C)	87% 3.5 ± 1.2 <sup>b</sup>	20% 2.5 ± 0.4 <sup>a</sup>	100% 5.1 ± 0.8 <sup>c</sup>	93% 4.3 ± 0.8 <sup>c</sup>

\* In horizontal rows figures with different superscripts differ significantly ( $p < 0.05$ ).

TABLE III

The bacteriological condition of brain hygienically removed from *ritually slaughtered* calves ( $n = 30$ ), measured initially (day 1) and after 7 days of storage at  $3 \pm 1^\circ\text{C}$  (day 8); plates appropriate for enumeration (%), mean bacterial counts and standard deviation ( $\log_{10}$  cfu  $\text{g}^{-1}$ )

	Day 1		Day 8	
Aerobic colony count (3 days, $30^\circ\text{C}$ )	43%	$2.3 \pm 0.3^{a,*}$	100%	$5.6 \pm 0.9^b$
Aerobic colony count (14 days, $4^\circ\text{C}$ )	7%	$2.1 \pm 0.1^a$	100%	$6.1 \pm 0.9^b$
Enterobacteriaceae colony count (20 h, $37^\circ\text{C}$ )	0%	$< 1.8^a$	10%	$3.5 \pm 1.1^b$

\* In horizontal rows figures with different superscripts differ significantly ( $p < 0.001$ ).

In view of the similar initial contamination of hemispheres of ritually slaughtered and mechanically stunned animals one would expect to find negligible differences in contamination levels on day 8. Nevertheless Table III shows aerobic colony counts at  $30$  and  $4^\circ\text{C}$  to have increased to levels even higher than those observed in samples obtained by mechanical stunning ( $p < 0.01$  and  $p < 0.001$ , respectively), probably due to differences in composition of the initial contaminating microflora. Moreover, after one week of chilled storage, 10% of the plates became positive for Enterobacteriaceae which corresponded to a mean count of  $3.5 \log_{10}$  units. Although the counts were significantly lower compared to counts of the mechanically stunned group, recontamination with Enterobacteriaceae was not expected in view of the hygienic measures taken during removal of the brain.

These results prompted a survey on the composition of the Enterobacteriaceae flora from brains of kosher-slaughtered calves after one week of storage at  $3 \pm 1^\circ\text{C}$ . For this purpose all colonies obtained in VRBG plates were streaked onto plates with MacConkey agar incubated at  $37^\circ\text{C}$  and single colonies thus obtained examined for the traits of the main genera of Enterobacteriaceae by the procedures described earlier. In addition to assessing their psychrotrophic character, cultures from these colonies were made in brain heart infusion broth (BHI) (Oxoid CM 225) incubated at  $37^\circ\text{C}$  and subcultured on VRBG plates incubated at  $30$  as against  $43^\circ\text{C}$  (Mossel and Zwart, 1960).

83% of the colonies were confirmed to be Enterobacteriaceae. Table IV presents

TABLE IV

Distribution of genera (%) within the Enterobacteriaceae family isolated from calf brain, hygienically removed from ritually slaughtered calves ( $n = 30$ )

<i>Hafnia</i>	56
<i>Serratia</i>	24
<i>Erwinia</i>	16
'Alkalescens-Dispar'	4

\* Anaerogenic, non-motile variant of *Escherichia coli*.

the distribution of genera within these Enterobacteriaceae. All Enterobacteriaceae colonies were found to grow on VRBG agar at 30°C, but not at 43°C. With the exception of the *Alkalescens-Dispar* isolate they showed negative response in lactose dissimilation tests (24 h/30°C) and would consequently have been missed in conventional *coli-aerogenes* determinations.

The Enterobacteriaceae flora did not comprise genera of specific animal origin. This indicates that in the present experiment dispersion of bacteria via blood or faeces during or after removal of the brain from the skull had been successfully avoided. The finding that only VRBG surface plates incubated at 30°C showed any growth is in agreement with data of van der Zee et al. (personal communication) indicating that non-pathogenic Enterobacteriaceae will generally not grow at 43°C. Consequently the slight increase in the Enterobacteriaceae load in the present experiment is the result of contamination with environmental types of a psychrotrophic nature that are known to be harmless for man, with the exception of *Yersinia* species (Mossel, 1982).

Our findings illustrate once more the need to segregate enteric contamination of foods of animal origin from subsequent proliferation of bacteria of environmental origin (Mossel, 1982). These data also substantiate that brain tissue is extremely susceptible to bacterial spoilage under the conditions of chilled storage conventionally applied.

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