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BACTERIOLOGICAL QUALITY ASSURANCE OF PORCINE TONGUES.

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SUMMARY

INTRODUCTION
Porcine tongues are mainly used for human consumption and generally incorporated in heat processed meat products.They are often visibly contaminated with blood, saliva, hair and dirt. Their keeping quality is limited. Bacteriological Quality Assurance (BQA) along the meat production-line has to rely on longitudinally intergrated Good Manufacturing Practices (GMP´s). So far, BQA concerning porcine tongues has appeared to be insufficient.The purpose of the described investigation is (a) to obtain information on the bacteriological quality of porcine tongues in the different stages of the slaughtering proces; (b) to elaborate GMP ´s for the production of porcine tongues.

MATERIALS AND METHODS
Line studies were carried out in sixteen slaughterhouses. Two investigators qualified the contamination grade of porcine tongues relying on fixed criteria, using a check list. At two plants a bacteriological linecontrol was carried out twice. At five stages of the slaughtering process i.e. after (a) bleeding (b) dehairing (c) evisceration (d) collection (e) centrifugation, samples of tongue mucosa were taken. The following colony counts per cm2 were assessed: mesophilic aerobic colony count, Enterobacteriaceae, Gram negative bacteria and Brochothrix thermosphacta. Histobacterioscopic examination was carried out, in order to assess the numbers and location of microorganisms in tongues. Experiments on an advanced centrifuge process under standardised conditions were involved in the investigation. The effects of time: (20s vs 40s), load:(10kg vs 15kg) and water expenditure:(20 l per min. vs 40 l per min.) on the bacteriological quality and cleaning efficiency were assessed. Aerobic colony counts and Enterobacteriaceae per cm2 tongue mucosa were determined and histobacterioscopic examination of the mucosa was carried out.

RESULTS
After stunning and bleeding the mucosa of the tongues was contaminated with dirt, blood and mucus. Contamination of tongues with stomach contents and damage of tongues took place during dehairing and polishing. The grade of the visual perceptible contamination depends on : (a) filling grade of the stomach (b) type of machinery.
Specific cleaning equipment in the slaughterline for tongues has hardly been developed. Cleanliness of the tongues was insufficient at 11 of the 16 plants. The highest bacteriological contaminaton of porcine tongues was found after bleeding. Mesophilic aerobic colony count, Enterobacteriaceae, Gram negative bacteria and Brochotrix thermosphacta were approx. 6.1, 2.9, 3.4 and 4.1 log10 N per cm2 respectively. Production stages reducing the contamination were scalding and the centrifuge process. The results of the bacteriological examinations were substanciated by the histobacterioscopic findings. By application of the advanced centrifuge proces a 100 fold reduction of the transient flora viz. Enterobacteriaceae and in optimal cleanliness of the mucosa of porcine tongues can be attained. Considering economic as wel as hygienic aspects a time, load, water expenditure ratio of 20 s/ 15 kg/ 20 l per min. is preferable.
GMP concerning BQA of porcine tongues includes
(a) slaughtering of pigs with an empty stomach only.
(b) avoiding contamination during evisceration
(c) application of a cleaning process, e.g. the centrifuge process.

INTRODUCTION

Porcine tongues are mainly used for human consumpt and generally incorporated into heat-processed me products. After slaughter they are often visi contaminated with blood, saliva, hair, and dirt. Th keeping quality is low. When they are used incorporation into meat products, tongues must clean and must have an acceptable bacteriologi quality.
Bacteriological Quality Assurance (BQA) of varie meats along the meat production-line has to rely longitudinally integrated Good Manufacturing Practic (GMP's). So far, BQA concerning porcine tongues proved to be insufficient. The purpose of investigation described below is (a) to obt information on the bacteriological quality of porc tongues in the different stages of the slaughteri process; (b) to elaborate GMP's for the production porcine tongues. The described experiments here w part of the GMP-development research programme variety meats of the Departement.

MATERIAL AND METHODS

Line studies
Line studies were carried out in sixteen pig slaugh houses. Two investigators assessed the contamina grade of porcine tongues, relying on fixed crite and using a checklist. The effect of cleaning processes was expressed in the following cod ++ = good, + = sufficient, - = insufficient, -- = ba

Bacteriological line control
At two of the sixteen plants (A, B) a bacteriologi line control was carried out twice. At five stages the slaughtering process, i.e. after (a) bleeding dehairing (c) evisceration (d) collection centrifugation 10 tongues were collected, and sampl of tongue mucosa were taken. With a cork bor (ϕ15 mm) one tissue sample per tongue was punched ou At each line control, tongues collected for sampli originated from one herd.
The following colony counts per cm² were assessed: mesophilic aerobic colony count: Tryptone Glucose Be Extract Agar (TGEA, Difco 002.01 3 d at 30°C). Enterobacteriaceae: Violet Red Bile Glucose Ag (VRBG, Oxoid CM 485, 1 d at 37°C) (3).
Gram-negative bacteria: Olson's medium (3 d at 20° (5).
Brochothrix thermosphacta: Gardner's medium (3 d 20°C) (1).
In order to get an impression of the distribution of the numbers of micro-organisms on tongu histobacterioscopic examination was carried out. each of the 5 stages of the slaughtering process samples of the tongue mucosa were taken with scalpel. Per sample two paraffine sections, thick, were cut perpendicularly to the surface, stained with heamatoxylin and eosin and Löffle methylene blue. A classification scheme for presence of bacteria was used. The following cod (magnification 400x) were used: + = >100 bacteria field of view, +/- = 1-100 bacteria per field - = bacteria per field (number of bacteria calculated the mean of 4 counted fields).
The bacteriological contamination of stomach conte and scalding water was investigated. At each li control two samples of 100 ml of water were taken o of the scalding tank. Stomach contents were examin from 5 pigs with an almost empty stomach and fro pigs with a full stomach. In both examinatio mesophilic aerobic colony counts and Entero bacteriaceae were assessed.

Centrifuge process
Experiments on a centrifuge process working by t same principle as found in the line studies, un standardized conditions were included in

investigation. A centrifuge consisting of a fixed drum with a rotating bottom (®Selo 750 RPM, ϕ600 mm, content 60 l) originally designed for the cleaning of tripes was used. During rotation tongues are intensively rinsed with water. The effects of centrifugation time (20 s vs 40 s), load (10 kg vs 15 kg), and water expenditure (20 l per min. vs 40 l per min.) were assessed. Mesophilic aerobic colony counts and Enterobacteriaceae (per cm² tongue mucosa) were determined before and after centrifugation. A histobacterioscopic examination of tongue mucosa was carried out. The visually perceptible contamination grade of the tongues was also evaluated.

Statistical analysis of data

Colony counts were expressed in colony forming units (cfu) per cm² of tissue and then transformed into log values. To determine the significance of the differences between counts these were submitted to an analysis of variance. Samples with less than 7 colonies on the first decimal dilution plate and therefore inappropiate for colony counting (2) were assigned counts corresponding to the statistical limits of detection.

RESULTS

Line studies

After stunning and bleeding, the muscosa of the tongues was contaminated with dirt, blood and mucus. During scalding at 62°C the surface layer of the mucosa of the tip of the tongues became detached. Contamination of the tongues with stomach contents and dirt from the machinery took place during dehairing and polishing. Damage of the tongues by the machinery also took place at these stages. The grade of the visually perceptible contamination depended on (a) fulness of the stomach (b) type of machinery (c) cleanliness of the machinery. During evisceration tongues were contaminated by contact with the apron of the worker. In most slaughter houses specific cleaning equipment for tongues in the slaughter line had hardly been developed. The cleaning effect of showering, washing, and centrifugation are summarized in Table 1. Cleanliness of the tongues at the end of the slaughter line was far from optimal at 11 of the 16 plants.

Bacteriological line control

The results of the bacteriological line control are presented in Fig. 1 and Tables 2, 3, and 4.

Fig. 1 and Table 2 show that the highest bacteriological contamination of porcine tongues was found after bleeding. Mesophilic aerobic colony count Enterobacteriaceae, Gram-negative bacteria, and Brochothrix thermosphacta were approx. 6.1, 2.9, 3.4 and 4.1 log10 N per cm² respectively. A significant decrease of colony counts was found after dehairing. After evisceration an increase in the mesophilic aerobic colony counts of tongues was found. After collection at slaugterhouse A a decrease and at slaughterhouse B an increase in colony counts were observed. The effect of the centrifuge process at slaughterhouse A was less effective than that at slaughterhouse B (especially at day 1). At day 2 at slaughterhouse A and days 1 and 2 at slaughterhouse B a significant decrease in the mesophilic aerobic colony counts and Enterobacteriaceae after centrifugation was observed. Following the processing stages, a decrease in Brochothrix thermosphacta was found. Colony counts for Gram-negative bacteria of the tongues varied, no tendency to decrease was observed. In scalding water Enterobacteriaceae were found to be below the detection level (<1.8). The mean value for mesophilic aerobic colony count was 2.4 ± 0.4.

Histobacterioscopic examination shows that in all stages of the slaughtering process >100 bacteria (score +) per field of view of a section of the mucosa were found.

An analysis of variance of the bacteriological data (Table 3) shows that there was a significant two-way interaction between stage of the slaughtering process and sampling day for Enterobacteriaceae and for Gram-negative bacteria. Apart from the two-way interactions there was mostly a significant main effect caused by sampling day for Enterobacteriaceae, Gram-negative bacteria, and Brochothrix thermosphacta. Stage of the slaughtering process was the only variable which caused a highly significant one-way variance for every bacteriological parameter.

In Table 4 are presented pH values and colony counts for stomach contents of 10 pigs. Six samples contained high colony counts for Enterobacteriaceae (>5.8) Below a pH of 3.5 (of the stomach contents) Enterobacteriaceae were found to be below the detection level (<2.8).

The results of the centrifuge experiments under standardized conditions are presented in Tables 5, 6, and 7.

Table 5 shows that the centrifuge process caused a significant decrease in the mesophilic aerobic colony counts and Enterobacteriaceae of the tongues.

No significant differences were found between colony counts caused by different centrifugation times, loads, and water expenditures. Only the long centrifugation time (40 s) resulted in lower numbers for mesophilic aerobic colony counts (Table 6).

Table 7 shows significant two-way interactions of variance for the mesophilic aerobic colony counts but not for Enterobacteriaceae. All the combinations used resulted in the same visually assessed cleaning efficiency, e.g. ++ = Good.

Histobacterioscopic examination showed that dirt and adherent bacteria adhering to the surface were removed. The colonized bacteria on the mucosa were still not removed. The presence of bacteria was classified in all the cases as +. (>100 bacteria per field).

DISCUSSION AND CONCLUSIONS

During transport of pigs to and storage at the slaughter house tongues are contaminated by the intake of dirt, faeces, and water. Pigs are slaughtered in a hanging position, as a result of which contents of the upper digestive tract, mucus, and dirt flow onto the tongues. This explains the high contamination grade of tongues after bleeding. During scalding in a tank at 62°C, thermolabile Gram-negative bacteria like Enterobacteriaceae are killed. (See colony counts for scalding water.) Decontamination of the tongues takes place during scalding because the mouth is open. The strong sphincter of the stomach does not allow any contents to flow towards the throat. During dehairing and polishing a strong pressure is exerted on the carcass, e.g. on the stomach, especially when it is full. The sphincter may open a little and contents of the stomach may flow towards the throat and contaminate the tongue, the machinery, and the carcass. Stomach contents may contain high numbers of bacteria and even Enterobacteriaceae. The increase in bacteriological contamination after evisceration is caused by the polishing machinery, stomach contents, and contact with the aprons of the workers during evisceration. The decrease in bacterial colony counts after collection at the slaughterhouse B is probably caused by the use of water during collection. At slaughterhouse B the visual cleanliness of the tongues after centrifugation was better. This explains the differences in results between the assessed colony counts for tongues sampled at slaughterhouse A and slaughterhouse B. From the results of the standardized centrifuge process it is clear that dirt and stomach contents can be removed effectively. Independent of the combination of centrifugation time, load, and water expenditure there is a significant decrease in

transcient bacteria, but not in the initial flora. These findings are substantiated by the histobacterioscopic findings. From the literature it is well known that colonized bacteria are strongly attached to the tissue (4).
Nevertheless, experiments with veal tongues showed that it is possible to remove colonized bacteria from the mucosa by the use of 2% v/v lactic-acid dilution during centrifugation (6). Considering economic as well as hygienic aspects, a time, load, water expenditure ratio of 20 s/15 kg/20 l per min. is preferable.
Finally, it can be concluded that GMP concerning BQA of porcine tongues must include:
(a) slaughtering of pigs with an empty stomach only.
(b) cleanliness of the machinery (easy to clean and disinfect).
(c) avoiding contamination during evisceration.
(d) application of a cleaning process to the tongues, e.g. a centrifuge process.

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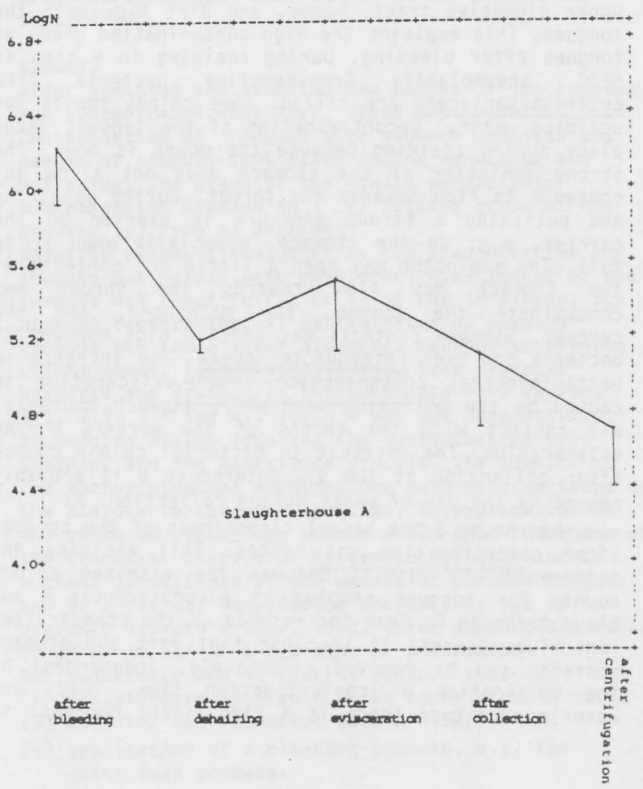
Fig 1. Mesophilic aerobic colony count (in $Log_{10}N/cm^2$ and standard deviation) of porcine tonques at different stages of the slaughtering process.



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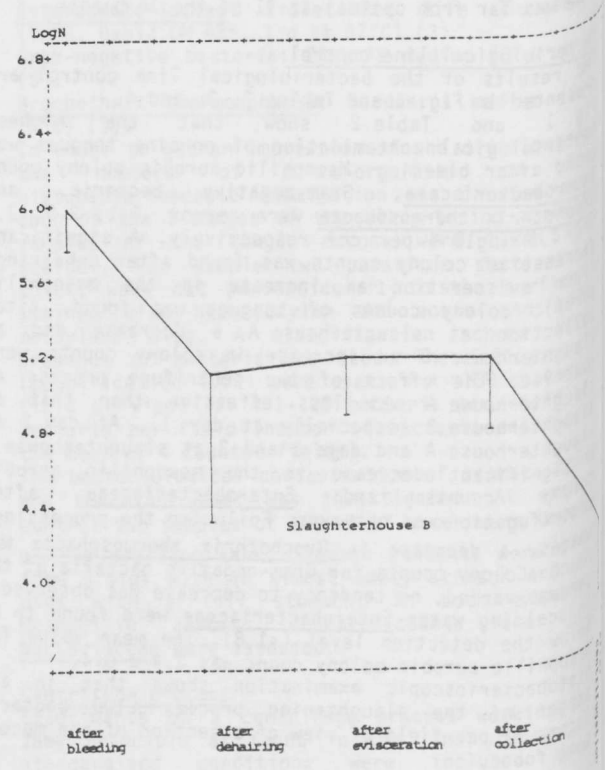

TABLE 1. Visually perceptible cleaning effects on the contamination grade of porcine tongues at 16 slaughterhouses.

	Processing			Total result
	Showering	Washing	Centrifugation	
1	--	-		-
2			+	+
3		-	-	-
4			+	+
5				--
6		-		-
7			++	++
8			-	-
9	--			--
10			+	+
11		--		--
12	-		-	-
13	-			-
14			-	-
15		--		--
16	+			+

++ = good
+ = sufficient
- = insufficient
-- = bad

TABLE 4. Colony counts (in Log_{10} Ng^{-1}) of stomach contents of 10 pigs in the slaughterline.

Pig*	pH	Mesophilic aerobic colony count	Enterobacteriaceae
1	5.6	7.7	7.3
2	5.4	8.0	7.5
3	3.9	7.4	7.3
4	3.3	5.9	<2.8
5	2.6	7.0	<2.8
6	6.9	7.1	5.8
7	6.7	7.6	6.9
8	5.5	7.4	6.9
9	3.5	4.9	<2.8
10	2.4	4.8	<2.8

*Pig 1 - 5 : full stomach
6 - 10 : almost empty stomach

TABLE 5. Effect of the centrifuge process on bacterial counts (in Log_{10} N per cm^2 and standard deviation) of porcine tongues.

	N	Mesophilic aerobic colony count	Enterobacteriaceae
unprocessed tongues	20	5.5 ± 0.4	3.8 ± 0.5
processed tongues	80	4.5 ± 0.5	2.0 ± 0.5
significance		p<0.0001	p<0.0001

The statistical procedure used was an analysis of variance.

TABLE 2. Bacterial counts (in $Log_{10}N/cm^2$ and standard deviation) of porcine tongues at different stages of the slaughtering process.

slaughter house	Day	STAGE	N	Mesophilic aerobic colony count	Enterobacteriaceae	Gramneg.bacteria	Brochothrix thermosphacta
A	1	After bleeding	10	6.1 ± 0.4	2.9 ± 0.3	4.0 ± 0.6	4.2 ± 0.2
		After evisceration	10	5.5^{b} ± 0.4	2.3^{d} ± 0.4	3.1^{d} ± 0.4	3.1^{d} ± 0.8
		After collection	10	5.0^{a} ± 0.4	2.3 ± 0.5	3.0 ± 0.3	2.9 ± 0.3
		After centrifugation	10	4.7 ± 0.3	2.3 ± 0.3	3.9^{c} ± 0.7	2.9 ± 0.5
A	2	After bleeding	10	6.4 ± 0.3	3.0 ± 0.7	2.9 ± 0.2	4.1 ± 0.5
		After dehairing	4	5.1^{d} ± 0.2	2.0^{a} ± 0.3	2.9 ± 0.4	2.9^{c} ± 0.5
		After collection	10	5.2 ± 0.4	2.6^{a} ± 0.5	3.0 ± 0.3	2.7 ± 0.2
		After centrifugation	10	4.7^{b} ± 0.3	$<1.6^{d}$	3.2 ± 0.3	<2.6
B	1	After bleeding	10	6.0 ± 0.2	3.0 ± 0.3	3.2 ± 0.3	4.3 ± 0.5
		After dehairing	10	4.8^{d} ± 0.3	2.4^{c} ± 0.4	$<2.6^{d}$	$<2.6^{d}$
		After collection	10	5.2 ± 0.4	3.2^{d} ± 1.0	3.8^{d} ± 0.7	3.2^{d} ± 0.4
		After centrifugation	10	4.4^{d} ± 0.3	$<1.6^{d}$	3.8 ± 0.3	2.7^{d}
B	2	After bleeding	10	6.0 ± 0.2	2.7 ± 0.3	3.6 ± 0.3	3.9 ± 0.6
		After evisceration	10	5.2^{d} ± 0.3	2.5 ± 0.6	3.2^{a} ± 0.5	2.8^{d} ± 0.3
		After collection	10	5.3 ± 0.4	2.7 ± 0.7	3.6 ± 0.5	2.8 ± 0.4
		After centrifugation	10	4.0^{d} ± 0.5	$<1.6^{d}$	2.9^{c} ± 0.4	<2.6

Colony counts at each stage are only compared with colony counts at the preceding stage.
Statistics were carried out with analysis of variance.
a = $p < 0.05$ b = $p < 0.01$
c = $p < 0.005$ d = $p < 0.001$

TABLE 3. Significance in variance analysis of: Mesophilic aerobic colony counts, Enterobacteriaceae, Gram negative bacteria and Brochothrix thermosphacta of porcine tongues at two slaughterhouses, five stages of the slaughtering-process and two sampling days.

	DF	Mesophilic aerobic colony count		Enterobacteriaceae		Gramnegative bacteria		Brochothrix therm.	
Slaughterh.		1	2	1	2	1	2	1	2
two-way var. stage / day	2	ns	ns	p<0.001	ns	p<0.005	p<0.001	ns	ns
one-way var. stage	4	p<0.001	p<0.001	p<0.001	p<0.001	p<0.005	p<0.001	p<0.001	p<0.001
day	1	ns	ns	ns	p<0.05	p<0.001	p<0.05	ns	p<0.01

DF = Degrees of Freedom
ns = not significant

TABLE 6. Effect of (a) water expenditure (b) centrifugation time (c) load in the centrifuge process on bacterial counts (log N per cm and standard deviation) of porcine tongues.

	N	Mesophilic aerobic colony count	Enterobacteriaceae
a 20 l	40	4.4 ± 0.4	1.9 ± 0.4
40 l	40	4.6 ± 0.6	2.2 ± 0.6
sign.		N.S.	N.S.
b 20s	40	4.6 ± 0.6	2.1 ± 0.5
40s	40	4.4 ± 0.5	2.0 ± 0.5
sign.		p<0.05	N.S.
c 10 kg	40	4.5 ± 0.6	2.1 ± 0.6
15 kg	40	4.5 ± 0.5	2.0 ± 0.5
sign.		N.S.	N.S.

N.S. = not significant

The statistical procedure used was an analysis of variance.

TABLE 7. Two- and three-way interaction in an analysis of variance on water expenditure, centrifugation time, and load on bacterial counts of porcine tongues in the centrifuge process.

Interactions of the variables	Mesophilic aerobic colony count	Enterobact.
Waterexpenditure-centrifugation time-load	N.S.	N.S.
Waterexpenditure-load	p<0.05	N.S.
centrifugation time-load	p<0.01	N.S.
Waterexpenditure-centrifugation time	p<0.01	N.S.

N.S. = Not Significant