Information Note

Background for the Danish verification programme of the microbiological quality of stomachs, casings and toes

19 July 2011
Proj. No. 2000209
Version 01
HCH/MOL/LHAN

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Introduction

Background

As a prerequisite for approving Danish establishments as exporters of pork to the Peoples Republic of China, a verification programme ensuring the microbiological quality of stomachs, casing and toes was developed by DMRI including microbiological criteria.

This prerequisite programme is now part of the Danish export legislation.

It should be noted that microbiological analyses are not suitable as directly process control, since there is too long time until the result is ready. The on-going control must therefore be based on other parameters as for example temperature measurements and visual inspection of absence of contaminations.

Microbiological analyses are suited as documentation of the bacteriological quality and for verification purposes.

In this report the background for the Danish verification programme of the microbiological quality of stomachs, casings and toes is described.

The aim was to develop a programme based on ”what can be achieved when using Good Manufacturing Practices”.

Toes

In Danish pig slaughterhouses samples are routinely collected from random selected carcasses and analysed for Total Viable Counts (TVC) and E. coli.

TVC is an expression of the general load of bacteria on the carcass.

The number of E. coli is an expression of the amount of faecal contamination on the carcass.

Toes for human consumption must be free of bristles, oil and other contaminations. The toes are part of the carcass and should therefore meet the microbiological requirements for the carcass.

This was the background for choosing the same limits for toes as is used as microbiological verification of Danish pig carcasses, see below.

In order to verify the microbiological quality of toes specifically, it was decided weekly to collect 5 random toes from one day of slaughter and analyse them individually for TVC and E. coli.

5 samples per week are sufficient for verification purposes. If the results are satisfying during a 6 weeks period, the sampling frequency can be reduced to once every second week.

Sampling and analyses

Samples are collected by swabbing the whole toe with a sterile gauze pad, which is moistured in 10 ml sterile buffered peptone water.

The samples are analysed for TVC and E. coli with a method approved by the Danish Veterinary and Food Administration (DVFA) (reference methods: ISO 4833 for TVC and AOAC 991.14 for E. coli).

Handling of results

Based on an average area for toes the TCV/cm^2 and the number of E. coli per cm^2 are calculated. The average area for the back toe and front toe are 374 cm^2 and 233 cm^2 respectively under the following conditions

- The area between the hoofs is not swabbed
- The back toe is divided from the ham by sawing in the meta-tarsal bone close to its dorsal end and before the joint
- The front toe is divided from the fore end in the joint closest to the fore end of the carcass

An average is calculated for the 5 samples (5 toes) after log-transformation of the counts (log to the base of 10). The log-transformation is done in order to get data that is normally distributed (Niemelä, 1983).

In case numbers of E. coli is below the detection limit, a value of -1.5 log cfu/cm^2 is used in the calculation of the mean.

In case numbers of TVC is below the detection limit, a value of 0 log cfu/cm^2 is used, if the lowest dilution is 10^{-1} in the dilutions series used for plating, a value of 1 log cfu/cm^2 is used if the lowest dilution is 10^{-2}, and 2 log cfu/cm^2 if the lowest dilution is 10^{-3}. This ensures that the used value is higher than the detection limit depending on the used dilution, which will ensure, that the calculated average does not underestimate the true average.

The calculated average is evaluated in relation to the microbiological criteria below.

	TVC **log/cm^2**	***E. coli*** **log/cm^2**
Acceptable interval	<3.6	<0.1
Marginal interval	3.6 - 4.6	0.1 - 1.1
Unacceptable interval	>4.6	>1.1

Concerning the limits and how they were developed, please see the attached report: Comparison of results from the bacteriological monitoring of slaughter hygiene with the EU and USA methods at slaughter of pigs", 19187.1.

It should be noted that the reason for the Danish preference for using E. coli is that E. coli is a better indicator of faecal contamination compared to e.g. Enterobactericeae and besides this the requirement on using E. coli (carcasses) is within the US legislation, and this requirements has been implemented in US-approved Danish pig slaughterhouses for many years.

Follow up

A result in the unacceptable interval means that the slaughter process must be examined due to faecal contamination and slaughter hygiene, if possible the cause must be found and reoccurrence prevented.

Fresh stomachs

Stomachs for human consumption must be free of visible digestive content.

Also in this case the microbiological verification was based on weekly collection of 5 samples. The samples are collected at random from one day of production of stomachs that are cleaned and ready for packaging.

5 samples per week are sufficient for verification purposes. If the results are satisfying during a 6 weeks period, the sampling frequency can be reduced to once every second week.

TVC was chosen as a parameter for the general bacteria load, e.g. whether the stomachs were properly chilled after cleaning, and the number of E. coli as a measurement for how effective the stomachs were cleaned for digestive content.

Sampling and analyses

From each stomach minimum 10 g is collected, which is transferred to a sterile bag together with sterile peptone water and homogenised by stomaching.

The samples are analysed for TVC and E. coli with methods approved by DVFA (reference methods: ISO 4833 for TVC and AOAC 991.14 for E. coli).

Handling of results

Results are reported as cfu (colony forming units) per g. After log-transformation (log to the base of 10) an average is calculated for the five samples.

Only limited data was available from studies of fresh stomachs, when the verification program was initiated.

Therefore a study was performed of “what could be achieved when using Good Manufacturing Practices”.

The study comprised 100 stomachs from 13 slaughterhouses and shoved that when using good manufacturing practises the counts of fresh stomachs will be within the acceptable interval in the microbiological criteria shown below.

	TVC	*E. coli*
	log/g	**log/g**
Acceptable interval	<6.5	<3
Marginal interval	6.5 - 7.5	3 - 3.5
Unacceptable	>7.5	>3.5

Follow up

In case of unacceptable results, the slaughter process must be evaluated in relation to faecal contamination and hygiene, if possible the cause must be found and reoccurrence prevented.

Cured casings

Cured casings must be free of visible faecal material.

Cured casings are a preserved product, and the number of bacteria will be reduced over time. Studies of how fast E. coli was reduced when curing casings showed that far the greatest reduction happened during the first 3 weeks (Christensen, 2002).

Therefore verification samples should be collected after minimum 3 weeks of curing, in order for bacteria numbers to reflect the general bacteria load and the amount of left over faecal material and whether the casings are thoroughly cured.

Sampling and analyses

5 samples are collected weekly. 5 samples per week are sufficient for verification purposes. If the results are satisfying during a 6 weeks period, the sampling frequency can be reduced to once every second week.

The samples are collected at random from one or more days of production, making sure that all samples derive from products being cured for at least 3 weeks.

From each sample minimum 10 g are taken. Excessive salt is removed, and the samples are transferred to sterile bags together with sterile peptone water and homogenised by stomaching.

The samples are analysed for TVC and E. coli with a method approved by the DVFA (reference methods: ISO 4833 for TVC and AOAC 991.14 for E. coli).

Handling of results

Results are reported as cfu per g. After log transformation (log to the base of 10) an average is calculated for the 5 samples.

The European Natural Sausage Casings Association (ENSCA) has in 1996 given recommendations for counts in cured casings (Fischer og Krol, 1997). They state that TVC <100,000 cfu/g is fully acceptable with a maximum value of 5,000,000 cfu/g. As indicator for faecal contamination Enterobacteriaceae is used in the recommendations, where an acceptable number is <100 cfu/g and a maximum number is 10,000 cfu/g.

The number of E. coli will be lower than the number of Enterobacteriaceae; in case of carcasses studies have shown that the prevalence will be app. 0.5 log-units lower, which corresponds to a factor 3 lower (Christensen, 2003).

This means that a fully acceptable number of E. coli in cured casings is <30 cfu/g with a maximum number of E. coli of 3,000 cfu/g in individual samples.

If the results are evaluated on an average of 5 samples, and with calculations as described earlier including handling of results below the detection limit, then the TVC should in average be <5 log cfu/g, and the number of E. coli should be less than 1.5 log cfu/g.

Based on this, the following microbiological criteria was developed:

	TVC **log/g**	*E. coli* **log/g**
Acceptable interval	<5	<1.5
Marginal interval	5 - 6.5	1.5 - 3.5
Unacceptable	>6.5	>3.5

Follow up

A result in the unacceptable interval means that the process must be examined due to faecal contamination and hygiene, if possible the cause must be found and reoccurrence prevented.

References

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