

Microbacterium thermosphactum: Fäulnisindikator für Rindfleisch

SIEGER J. MULDER

Zentralinstitut für Ernährungsforschung - TNO, Zeist, Niederlande

Im Zentralinstitut für Ernährungsforschung TNO wurden viele Untersuchungen ausgeführt in Bezug auf die Kontamination und das Wachstum von Bakterien auf Rindfleisch. Die Resultate dieser Untersuchungen werden kurz besprochen an Hand von Angaben über *Microbacterium thermosphactum*.

Die Kontamination von Rindfleisch mit *M. thermosphactum* stammt von der Haut, auf welcher sie immer angetroffen wird. Auf der Rinderhaut korrelieren die Mengen *Pseudomonas*bakterien und die Mengen *M. thermosphactum* positiv mit einander. Desinfektion der Haut von Schlachttieren senkt den Kontaminationsgrad des Rindfleisches mit psychrotrophen Organismen.

Bei Aufbewahrung von Rinderschlachtkörpern in Schlachthöfen steigt die Anzahl von *M. thermosphactum* bis mehr als $10^6/\text{cm}^2$ Bakterien in 10 bis 17 Tagen. Die wichtigsten Fäulnisorganismen, nämlich die *Pseudomonas*bakterien, erreichen diese Zahl einige Tage früher.

Bei hängendem Transport von Schlachtkörpern sind der Lastkraftwagen und das beförderte Fleisch wichtige Kontaminationsquellen. Beim Transport von Teilstücken fördert die Kreuzkontamination mit anderem Fleisch die Keimzahlen. *M. thermosphactum* und *Pseudomonas*bakterien sind meistens stark vertreten in der Kontaminationsflora.

Bei dem Versand und weiterer Verteilung von Rindfleisch wächst *M. thermosphactum* in einzelnen Fällen schneller als *Pseudomonas*bakterien, zum Beispiel bei Fleisch aufbewahrt in einer Gasatmosphäre von CO_2 und O_2 . In diesen Fällen entsteht Fäulnis (Verfärbungen, Geruchsabweichungen) hauptsächlich durch *M. thermosphactum*.

Microbacterium thermosphactum: spoilage indicator of beef

SIEGER J. MULDER

Netherlands Centre for Meat Technology, Dept. of Central Institute for Nutrition and Food Research-TNO

In the Central Institute for Nutrition and Food Research TNO extensive studies have been carried out on contamination and growth of bacteria on beef. The principal results of these investigations are reported and illustrated with data from analysis of *Microbacterium thermosphactum*.

M. thermosphactum is important for beef because of its psychrotrophic properties and its rapid growth on refrigerated beef. It was found on the skin of animals before slaughter. On these skins a positive correlation existed between the counts of *M. thermosphactum* and other psychrotrophic bacteria. It was shown that bacteriological contamination of carcasses for the greater part originates from the skin of animals. During storage of beef carcasses in slaughterhouses *M. thermosphactum* counts increase from about the limit of detection (10^2 bacteria per cm^2) after slaughter to ca 10^6 bacteria per cm^2 after 10 - 17 days. During this storage time some signs of spoilage become evident; this spoilage mainly resulted from growth of pseudomonads. During shipment of carcasses bacteriological contamination mainly related with the hygienic situation in transport vehicles and with microbiological condition of other meats. During shipment of primal cuts cross-contamination between the cuts is the cause of important changes in bacteriological condition. *M. thermosphactum* and pseudomonads represent an important part of the contaminating flora.

In some cases of meat distribution *M. thermosphactum* multiplies more rapidly than all other psychrotrophic bacteria. This is illustrated by results from research on beef stored in a gas atmosphere consisting of carbondioxyde and oxygen. In all these cases spoilage of meat (discolouration, taints) is principally caused by a high number of cells of *M. thermosphactum*.

B 4:2

Microbacterium thermosphactum: indicateur de détérioration dans la viande de boeuf

SIEGER J. MULDER

Institut Central de la Nutrition et de l'Alimentation - TNO, Zeist, Pays Bas

A notre Institut on a fait des recherches étendues sur la contamination et la croissance de bactéries dans la viande de boeuf. Les principaux résultats de ces investigations ont été rapportés et illustrés par des données d'analyse de *Microbacterium thermosphactum*. Nous avons trouvé *M. thermosphactum* sur la peau d'animaux avant l'abattage. Sur ces peaux les nombres de *M. thermosphactum* avaient une corrélation positive à d'autres bactéries psychrotropiques. La contamination bactériologique des cadavres était pour la plupart originaire de la peau des animaux. Pendant le stockage des cadavres de boeuf dans les abattoirs, le nombre de *M. thermosphactum* augmente à partir de la limite de détection (10^2 bacteria le cm^2) après l'abattage jusqu'à environ 10^6 bacteria le cm^2 après 10/17 jours. Pendant le transport des cadavres, la contamination bactériologique dépendait pour la plus grande partie de la situation hygiénique dans les véhicules de transport, et de la condition microbiologique d'autres produits carnés se trouvant dans les mêmes véhicules. Pendant le transport de morceaux coupés pour la première fois, la contamination croisée parmi les morceaux est la cause de changements considérables de la condition bactériologique.

Dans certains cas de distributions de viande, *M. thermosphactum* se multiplie plus rapidement que toutes les autres bactéries psychrotrophiques, ce qui est illustré par les résultats des recherches sur le boeuf stocké dans un atmosphère de gaz se composant de carbondioxyde et d'oxygène. Dans tous ces cas la détérioration de la viande (décoloration, odeur) est surtout causé par un grand nombre de cellules de *M. thermosphactum*.

Microbacterium thermosphactum - показатель порчи говядины.

С. И. МУЛДЕР.

из отдела "Нидерландский центр мясной технологии" Центрального НИИ питания и пищи, Зейст, Голландия.

В центральном НИИ питания и пищи при TNO проведены обширные исследования по загрязнении говядины и росте бактерий на ней.

Приводятся главные результаты, а также примеры из аналитических данных по *Microbacterium thermosphactum*. *M. thermosphactum* имеет значение для говядины из-за своих психротропических свойств и быстрого роста на охлажденном мясе.

M. thermosphactum было обнаружено на коже предубойных животных. На таких кожах существовала положительная корреляция между численностью *M. thermosphactum* и другими психротропическими бактериями. Было показано, что кожи являются главным источником бактериального загрязнения туш.

Во время хранения говядины в скотобойнях численности *M. thermosphactum* увеличиваются примерно от предела детекции (10^2 бактерий на cm^2) сразу после убоя, до примерно 10^6 бактерий/ cm^2 через 10-17 дней. В течение такого хранения обнаруживаются некоторые признаки порчи, обусловленной главным образом приростом псевдомонад.

Во время перевозки туш их бактериальное загрязнение зависит преимущественно от санитарных условий в фурагонах, а также от микробиального состояния других мяс.

Во время перевозки первичных отрезков взаимозаражение является источником значительных изменений в их бактериальном состоянии. *M. thermosphactum* и псевдомонады составляют существенную часть заражающей флоры.

При некоторых системах заготовки мяса *M. thermosphactum* размножается быстрее всех других психротропических бактерий, утверждением которого являются результаты наблюдений над говядиной, храняемой под атмосферой, состоящей из углекислого газа и кислорода. Во всех этих случаях порча мяса (изменения в краске, привкусы) была обусловлена главным образом повышенной численностью *M. thermosphactum*.

MICROBACTERIUM THERMOSPACTUM: SPOILAGE-INDICATOR OF BEEF

Sieger J. Mulder

Netherlands Centre for Meat Technology, Dept. of Central Institute for Nutrition and Food Research TNO
Zeist, The Netherlands

1. Introduction

In 1953 *Microbacterium thermosphactum* was described by McLean and Salzbacher (8) as a new species, isolated from fresh sausages. Since that time this bacterium was found by many investigators on various meat products.

M. thermosphactum forms chains of regular rods, is Gram-positive, catalase positive and grows aerobically at temperatures from 0-30 °C; it does not grow at 37 °C (2, 8, 15). Growth proceeds at oxygen concentrations of less than 0.2 % (15) and high carbon dioxide concentrations (14). Glucose and possibly glutamate are used up by growth on meat (5, 12). A selective medium has been developed by Gardner (4).

Relatively little is known of contamination of meat with *M. thermosphactum* and its relation with other groups of psychrotrophic micro-organisms. Not much has been published about its growth on meat under industrial conditions, and its contribution to spoilage either. In this paper these aspects are illustrated by investigations performed in 1972-1975.

2. Experiments, results and discussion

2.1 Bacteriological contamination of bovine carcasses

Psychrotrophic bacteria are found on bovine carcasses immediately after slaughter. Among these bacteria *M. thermosphactum* and pseudomonads have an important place.

It was investigated whether these bacteria could be found in bovine animals on the skin or in the digestive tract. From 5 animals samples of skin were taken after bleeding and from these the micro-organisms were washed with dilution fluid (7). After evisceration samples were taken too from different places of the contents of the digestive tract.

Counts of *M. thermosphactum* (4), pseudomonads (6) and aerobic count were performed from all samples, and the colonies of the selective media were confirmed.

The results show that *M. thermosphactum* is found on the skin of all 5 animals (table 1).

The number of these bacteria is rather small, e.g. 10^2 à 10^4 per cm^2 . Distinctly more pseudomonads are found on the skins although these bacteria only compose a minor fraction of the total microflora (fig. 1).

A significant positive correlation exists between the counts of *M. thermosphactum* and pseudomonads on the skin (rank-correlation of Spearman pL 0.05).

Fig.1. Mean bacteriological counts of cow skins

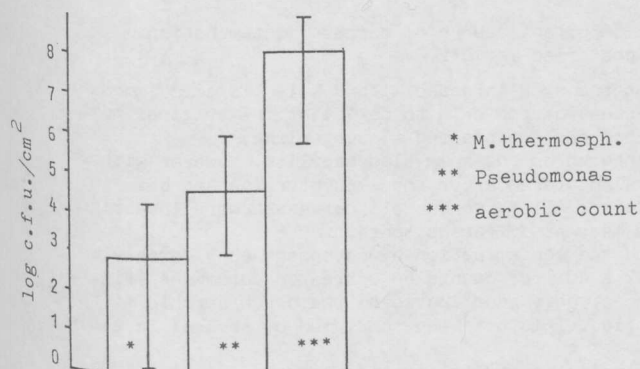


Fig.2. Aerobic counts on 6 spots of fattened calf carcasses

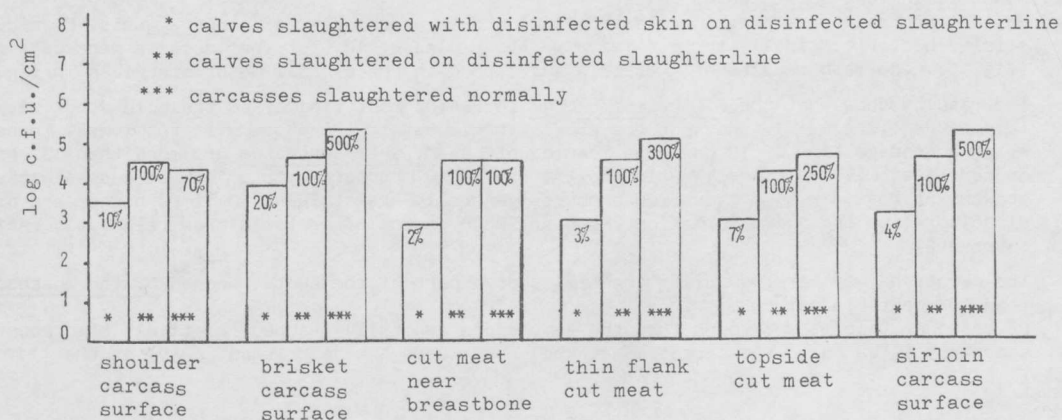


Fig. 3. Development of *M. thermosphactum* on bovine carcasses in 4 slaughterhouses

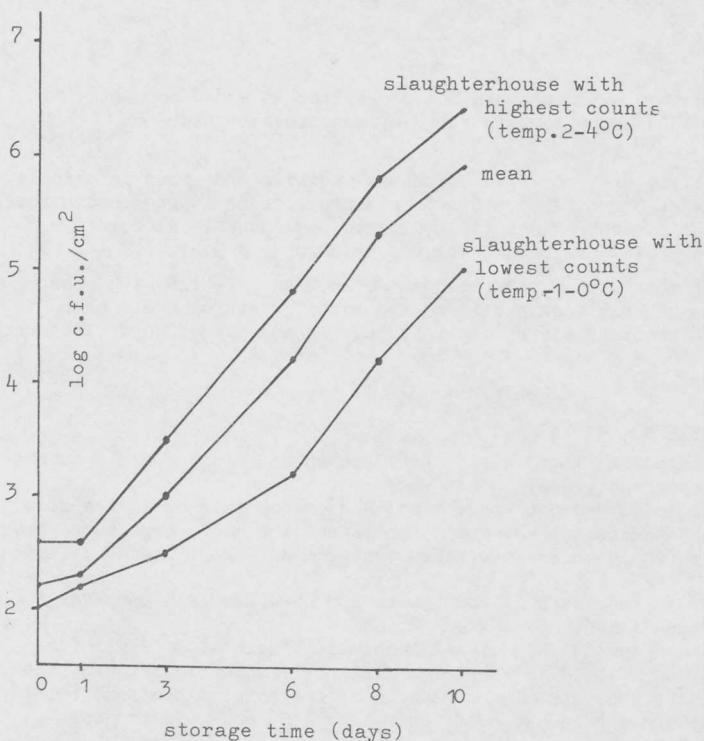
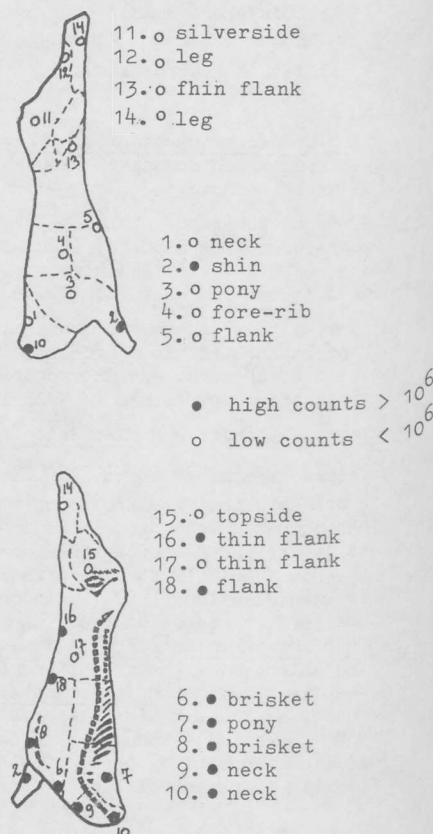


Fig. 4. *M. thermosphactum* on 18 spots of bovine carcasses after storage



Such a correlation is not found between the aerobic count and one of the psychrotrophic counts. In samples from the digestive tract *M. thermosphactum* has not been detected and pseudomonads only have been found incidentally in very low numbers.

All these results indicate that the bovine skin is an important source of carcass contamination. This has been confirmed by the following experiment, performed in 1972.

From two fattened calves the skin was cleaned with water and disinfected with 6 % lactic acid immediately before slaughter. This treatment reduced the aerobic count of the skin to ca 1 % of the original number of bacteria. Then both calves were slaughtered on a cleaned and disinfected slaughterline. By means of comparison two fattened calves were slaughtered on the same slaughterline, however without treatment of the skin. Also two fattened calves were slaughtered after the slaughterline had been in operation for 1/2 h and ca 30 calves had been slaughtered. All fattened calf carcasses were investigated bacteriologically by cutting 10 cm² from the surface meat at different spots. The results show that disinfection of the skin reduced the contamination of carcasses considerably. The aerobic count of treated calf carcasses was only 2 to 20 % of counts on untreated carcasses (fig. 2). From the aerobic count can be concluded that the skin not only contributes to the psychrotrophic microflora of freshly slaughtered carcasses, but also the mesophiles. This has been confirmed by several investigations (1, 9, 13).

Furthermore the results of fig. 2 shows that bacteriological contamination increases a little bit during operation of the slaughterline. After 1/2 h in operation the bacteriological load of some spots on the carcasses was 5 times higher than at the beginning.

2.2 Bacteriological growth on carcasses

Bovine carcasses were hung in cold stores of 4 slaughterhouses for about 2 weeks. Each carcass was sampled bacteriologically three times a week. Samples of 18 spots per carcass were cut from the surface (fig. 4). The mean results of extreme slaughterhouses and overall means are given in fig. 3.

The results show that the *M. thermosphactum* increases most rapidly in the cold store with the highest temperature (2 to 4 °C), and that the growth is slowest in the store with the lowest temperature (-1 to 0 °C). After a storage time of 10 days the mean counts of *M. thermosphactum* are more than 10⁶ bacteria per cm² on carcasses with relatively rapid growth, and ca 10⁵ bacteria per cm² on carcasses with relatively slow growth. At the same time pseudomonad counts generally are at least 10 times higher and often cause already discolouration and slime formation. However, both groups of bacteria grow relatively rapid on the same carcasses.

The carcasses were stored during the time that a part of the spots reached *M. thermosphactum* counts of more than 10⁶ bacteria per cm².

By means of this it was found that the same spots generally reached relatively high counts (fig. 4). These spots are situated on the carcasses at the neck, where the head is cut away, at the shin

of the foreleg, and on the cut meat of the breast and abdomen. All these spots are handled relatively intensive during slaughter by dehiding and cutting. This handling caused contamination with psychrotrophic micro-organisms from the skin, resulting in rapid growth on these spots of the carcasses (par. 2.1). Therefore proper handling methods during slaughter are very important in particular for these spots.

2.3 Contamination during shipment

Bovine carcasses and primal cuts were brought from slaughterhouse to butchery. This shipment took place in transport vehicles without refrigeration during 1/2 - 2 h. The experiments were performed in different seasons. The meat was investigated bacteriologically before shipment in the slaughterhouse and after shipment in the butcheries. Samples of 10 cm² were cut from the meat surface.

M. thermosphactum counts increase during shipment relatively much on meat with low bacterial counts (table 2A). Also the unhygienic condition of transport vehicles add to the bacteriological contamination (table 2B). It was found that contamination with M. thermosphactum cells generally was accompanied with contamination by pseudomonads. This connection between M. thermosphactum and pseudomonads is characteristic for the microflora of meat. Therefore cross-contamination between the various pieces of meat very likely causes bacterial increase. Sometimes the bacteria are transmitted because of unhygienic transport vehicles.

2.4. Growth on minced meat in gas atmosphere

Sometimes M. thermosphactum becomes the dominant organism of meat. This may occur when growth of pseudomonads is inhibited. A high portion of M. thermosphactum often is found in vacuum packed meat 1 à 2 weeks after packaging (14, 15), salted meat, meat stored in gas atmospheres consisting of carbon dioxide and oxygen, and in British fresh sausage (3).

Here the results of minced meat of 60 % beef and 40 % pork are given. This minced meat was packed under an atmosphere of 25 % carbon dioxide and 75 % oxygen and stored at 3 °C with a light intensity of 500 lux. After 3 days M. thermosphactum is the most numerous micro-organism of the microflora (fig. 5) and a count of 10⁷ bacteria per g is reached after ca 5 days. Thereafter discolouration soon begins, together with a deviating sour taint and, after boiling, an old meat taste. In a normal atmosphere minced meat spoils about twice as rapidly as in this gas atmosphere (10). Normal meat spoilage generally is accompanied with a pH increase; spoilage in gas atmosphere does not undergo a change in pH or even decreases it a little. This is also an indication that proteolytic pseudomonads are inhibited and the effect of M. thermosphactum has increased.

3. Conclusions

1. Contamination of carcasses with M. thermosphactum and other psychrotrophic micro-organisms principally originates from the skin of animals. Especially neck and cut meat from breast and abdomen are extensively contaminated.
2. During shipment bacteriological contamination occurs with a microflora adapted on meat. These micro-organisms are spread over the meat, among others by cross contamination and unhygienic condition of the transport vehicles.
3. The composition of psychrotrophic micro-organisms on meat adapts to outside influences. Inhibition of pseudomonads sometimes makes M. thermosphactum the dominating species.

4. Literature

1. Anon, Canadian industry takes stand on bacteriological standards. Nat. Prov. 1978, 4 febr., 8-24
2. Davidson, C.M. e.a., Some morphological and physiological properties of Microbacterium thermosphactum, J. appl. Bact. 31 (1968), 551-559
3. Dowdell, M.J., R.G. Board, The microbiological associations in British fresh sausages. J. appl. Bact. 34 (1971), 317-337
4. Gardner, G.A., A selective medium for the enumeration of Microbacterium thermosphactum in meat and meat products. J. appl. Bact. 29 (1966), 455-460
5. Gill, C.O., K.G. Newton, The development of aerobic spoilage flora on meat stored at chill temperatures. J. appl. Bact. 43 (1977), 189-195
6. Gyllenberg, J. e.a., A selective plating test for the demonstration of significant numbers of pseudomonads. Acta Agric. Scand. 10 (1960), 67-73
7. Int. Stand. Org. I.S. 2293.: Meat and meat products. Aerobic count at 3 °C (reference method)
8. McLean, R.L., W.L. Sulzbacher, Microbacterium thermosphactum spec. nov. A non-heat resistant bacterium from fresh sausage. J. Bact. 65 (1953), 428-433
9. Marot, A., Eine horizontale Schlachtlinie für Rinder. Schlachten Vermarkten 77 (1977) (9), 291-298
10. Mulder, S.J., Invloed van gasmengsels bestaande uit kooldioxide en zuurstof op de houdbaarheid van vlees. CIVO-report Nr. R. 3992 (Febr. 1973)
11. Mulder, S.J., B. Krol, Een onderzoek naar de bakteriologische gesteldheid van vers vlees. I. Tijdschr. Diergeneesk. 101 (1976) (11), 587-593; II. Tijdschr. Diergeneesk. 101 (1976) (11), 594-598; III. Tijdschr. Diergeneesk. 101 (1976) (23), 1306-1313
12. Newton, K.G., C.O. Gill, The development of the anaerobic spoilage flora of meat stored at chill temperatures. J. appl. Bact. 44 (1978), 91-94
13. Newton, K.G. e.a., Coliforms from hides and meat. Appl. Envir. Microbiol. 33 (1977) (1), 199-200
14. Sutherland, J.P. e.a., The effect of several gaseous environments on the multiplication of organisms isolated from vacuum packaged beef. J. Food Technol. 12 (1977), 249-255
15. Weidemann, J.F., A note on the microflora of beef muscle stored in nitrogen at 0 °C. J. appl. Bact. 28 (1965), 365-367.

B 4:6

Fig.5. Growth of micro-organisms in gas packed minced meat

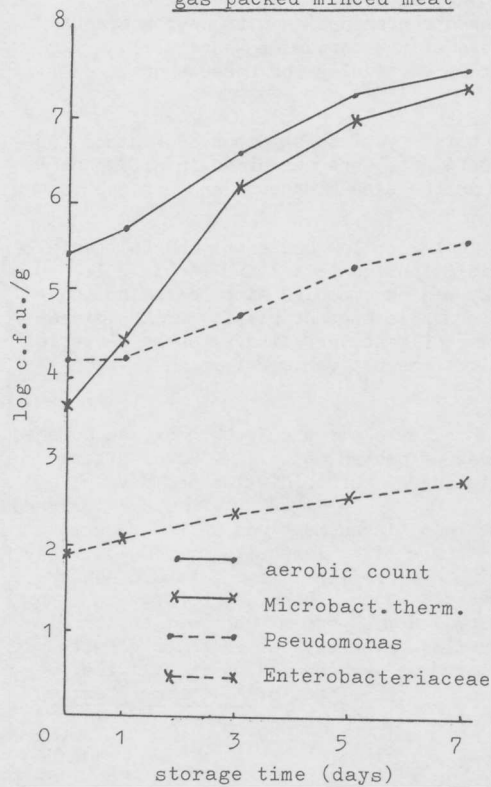


Table 1. Presence of M. thermosphactum on 6 spots of cowskins
M. thermosphactum determination x)

Cow nr.	1	2	3	4	5
skin sample:					
head	10 ²	10 ⁴	10 ²	-	10 ²
abdomen fore	10 ²	-	-	-	-
abdomen hind	-	-	10 ²	-	-
fore leg	10 ²	-	-	10 ³	-
hind leg	10 ²	-	-	-	-
tail	10 ²	10 ³	-	-	-

x) M. thermosphactum not determined (limit of detection 10²/cm²)

Table 2. Increase in M. thermosphactum counts on carcasses and cuts during shipment from slaughterhouse to butchery

2 A Effect of bacteriological load of meat

M. thermosphactum count before shipment (log/cm ²)	Increase of M. thermosphactum count on			
	carcasses		primal cuts	
	geometric mean (log)	extremes (log)	geometric mean (log)	extremes (log)
2.1 - 2.5	0.7	0.1-0.9		
2.6 - 3.0	0.7	0.3 - 1.0	0.8	0.0 1.7
3.1 - 4.0			0.6	0.0 - 1.2
4.1 - 5.0			0.3	0.0 - 0.8

2 B Effect of hygienic condition of transport vehicle

Hygienic condition transport vehicle	Increase of M. thermosphactum count on			
	carcasses		primal cuts	
	geometric mean (log)	extremes (log)	geometric mean (log)	extremes (log)
good	0.4	0.1 - 0.7	0.3	0.0 - 0.12
acceptable	0.7	0.3 - 0.9	0.4	0.0 - 0.9
bad	0.9	0.5 - 1.6	0.8	0.0 - 1.7