The official bacteriological meat examination in Germany with special regard to clostrida - present status and tendencies

H. EISGRUBER

Institute of Hygiene and Technology of Food of Animal Origin, Faculty of Veterinary Medicine, University of Munich, D-8000 München 22, Germany

SUMMARY: Red meat of slaughtered animals is commonly contaminated with low numbers (<10/g) of clostridia. The inoculation of only one hot boiling liver broth tube for detecting clostridia out of muscle-tissue as a part of the German official bacteriological meat examination is under discussion for a long while. In this context pilot studies were carried out to clarify the reisolation rate after mixing low numbers of 6 strains of clostridia into minced fresh meat. The liver broth tubes inoculated in a cool stage (20°C) showed an evident advantage for the reisolation. With the exception of C. sporogenes and one heat resistant type of C. perfringens less than 10 cells/gram meat could be reisolated. On the other side the reisolation procedure for clostridia from heat-treated liver broths showed the worst results. Evaluating the obtained results the use of three liver broth tubes which were not heated has been proposed.

INTRODUCTION: In 1986 the official bacteriological meat examination in Germany was revised. Actually the determination of clostridia was defined. The new established method should correspond with actual scientific knowledges and should be suitable for the routine investigation program. Consequently the new predicted method had to be a compromise as yet, because the positive report had to be fixed after a 48 hour-incubation-period. There are some good new proceeding-steps determining clostridia, but the inoculation of one "hot boiling" liver broth tube seems to be unsuitable. Commonly red meat of slaughtered animals is contaminated with low numbers of clostridia (<10/g), which mostly appear in the stage of vegetative growth (HOBBS et al., 1953; ZELLER, 1955; KRAUS, 1962; HALL and ANGELOTTI, 1965; SMART et al., 1979).

Additionally the distribution of low numbers of clostridia in red meat is irregular (NARAYAN, 1966; LEVETZOW, 1967). The own investigation-series confirmed this statement (EISGRUBER and REUTER, 1984; EISGRUBER, 1986). In this context pilot studies were performed clarifying the reisolation rate of low numbers of clostridia mixed into fresh minced pork due to the treatment of the inoculated liver broth tubes.

MATERIALS and METHODS: Out of the depth of muscle tissues of legally slaughtered pigs samples of 50-70 g portions were taken after decontaminating the surface of the meat of the scapular region by a hot iron. The mixing of muscle samples with defined numbers of clostridia were performed by an electrical mincer.

Of the 6 clostridia tested strains (origin see **table 1**) primary cultures were put into fluid thioglycolate media (MERCK 8190) which were incubated at 37°C for 18 hours. Afterwards culture fluid were centrifugated at 3.000 Upm for 10 minutes and the bacterial sediments were washed twice in NaCl-solution (physiol.). According to the McFarland standardization of turbidity measurement the visible density of the clostridia test cultures were adjusted to 10^7 microorganisms/ml and diluted to target inocula of 10^1-10^2 cells/ml. These inocula set by the McFarland standard were controlled by determining the anaerobic colony forming units per ml (cfu/ml) on surface plates of sheepblood agar by using the drop plating method (DIN-Standard 10161, part 2).

The 1 g inocula of minced pork containing a defined low number of clostridia were put into different treated liver broth tubes according to data from literature (EISGRUBER, 1986). The six tested modification treatments were performed in triple series (**table 1**). As control 1 g pieces of muscle taken directly out of the depth of the meat used for pilot studies were inoculated into three liver broth tubes (in a cool stage) to demonstrate the absence of clostridia originally.

RESULTS (table 1): The liver broth tubes inoculated in a cool stage (20°C and a chemical O_2-reduction method) showed an evident advantage for the reisolation of low numbers of clostridia. With the exception of C. sporogenes the reisolation of less than 50 cells/gram meat was possible. With another exception of C. perfringens NCTC 8798 less than 10 cells/gram could be reisolated.

On the other side the reisolation procedure for clostridia from tubes heated at 80°C for 5 minutes showed the worst results. The reisolation of C. perfringens was not possible and that of the tested C. sporogenes was very poor.

But two C. perfringens strains yielded complete growing in all other heat modifications of liver broth tubes if more than 50 cells/g meat were inoculated. Only the heat resistant type of C. perfringens showed reduced reisolation in liver broth tubes heated at 80°C.

The two tested C. bifermentans strains showed positive results in all combinations of liver broth tubes if more than 50 cells/gram meat were inoculated. Complete growing of C. sporogenes was possible if more than 100 cells/gram were proved.

DISCUSSION: Former investigations (KELLER et al., 1955; FRIESS and BUROW, 1980) pointed out that inoculated "hot boiling" liver broth tubes receive unfavourable detection rates of clostridia in comparison with such media inoculated at 80°C. Other studies showed much more positive results if the inoculation temperatures were fixed at 60°C or decreased at 20°C (ZELLER, 1955; REUSSE, 1982). These data confirm with the own results.

The use of so called "hot boiling" liver broth tubes for determining clostridia as regulated by the German official bacteriological meat examination is under discussion from another point of view. In laboratory practice the predicted inoculation temperature "hot boiling" showed a broad spectrum of "hot" temperatures which range between 80 and 95°C. For example experimental investigations had demonstrated a quick drop in temperature after having "hot boiling" tubes taken out of water baths for inoculation procedures (REUSSE, 1982).

The results of the pilot studies had shown positive reports of the complete 18 tubes tested if more than 1.0×10^2 clostridia per gram minced fresh meat were regular distributed. In case of C. perfringens, inoculated tubes which were heated at 80°C and held for 5 minutes at that temperature were negative, because of the absence of heat resistant spores. As noted before the German regulations predict the inoculation of one "hot boiling" liver broth tube. If there is such tube proved to be positive one can come to the conclusion that the inoculated 1 g piece of muscle contained a quantitiy of clostridia much more than >10/g or - it was only a lucky hit.

CONCLUSIONS: For detecting clostridia out of red meat you have to take into account that they are commonly irregular distributed in low numbers (<10/g) and mostly not sporulated. Therefore with regard to the own experimental results the following cultivation procedure for determining clostridia out of red meat has been proposed: Inoculation of three liver broth tubes in a cool stage (20°C) to dermine clostridia relevant to meat hygiene (e.g. C. perfringens, C. bifermentans, C. sporogenes) within the predicted 48-hours-cultivation-period.

That suggested method is not suitable for detecting sporulated or "slow growing" or even specialized clostridia (e.g. psychrophilic of thermophilic species).

The use of one hot boiling liver broth tube as predicted by the German official bacteriological meat examination is only practicable if clostridia are distributed in red meat in evident numbers (>100 cells/g).

At the end emphasis should be put on new and rapid cultivation media - for example the iron milk- or the Rapid Perfringens Medium (EISGRUBER, 1986). Probably the selective detection of C. perfringens should be taken more into account.

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Table 1: Reisolation of low numbers of 6 strains of clostridia (A-F) mixed into minced pork corresponding to numbers of confirmed positive liver broth tubes (triple tube estimation) after an incubation period of (24 h/48 h[a]) at 37°C

mixed strains of clostridia (cfu[b]/g minced pork)		treatment[c] of the inoculated liver broth tubes inoculum: 1 g minced (twofold) pork per tube					
		80/5	80/0	60/10	10/60	K	C
(A) C. perfringens type A heat resistant type 9 ACTC 8798							
$<10^1$	0.06×10^1	0	0	0	0	1	0
	0.2×10^1	0	0	0	0	1	0
10^1-10^2	2.2×10^1	0	0	0	0	3	3
	4.8×10^1	0	0	0	0	3	3
	5.0×10^1	0	1(2)	3	3	3	3
	8.3×10^1	0	1	3	3	3	3
$>10^2$	2.6×10^2	0	3	3	3	3	3
	3.1×10^2	0	2(3)	1(3)	2(3)	3	3
(B) C. perfringens type A NCTC 8238							
$<10^1$	0.7×10^1	0	0	0	0	3	3
10^1-10^2	1.5×10^1	0	0	0	0	3	3
	5.0×10^1	0	1(3)	1(3)	1(3)	3	3
	7.1×10^1	0	2(3)	2(3)	3	3	3
$>10^2$	1.5×10^2	0	3	3	3	3	3

(C) C. perfringens T 2/86 EISGRUBER (1986)							
$<10^1$	0.6×10^1	0	0	0	0	3	3
	0.8×10^1	0	0	0	0	3	3
10^1-10^2	1.3×10^1	0	0	0	0	3	3
	6.0×10^1	0	1(3)	3	3	3	3
	8.4×10^1	0	3	3	3	3	3
$>10^2$	1.3×10^2	0	0(3)	3	3	3	3
(D) C. bifermentans ATCC 9715							
$<10^1$	0.07×10^1	0	0	0	0	1	0
	0.7×10^1	0	0	0	0	3	1
	0.8×10^1	0	0	0	0	1	3
10^1-10^2	1.2×10^1	0	0	0	0	2	1
	1.8×10^1	0	0	0	1	3	3
	3.4×10^1	1(3)	3	3	3	3	3
	7.0×10^1	0(3)	3	3	3	3	3
	8.0×10^1	0(3)	3	3	3	3	3
$>10^2$	1.1×10^2	3	3	3	3	3	3
(E) C. bifermentans T 4/86 EISGRUBER (1986)							
$<10^1$	0.2×10^1	0	0	0	0	3	3
	0.5×10^1	0	0	0	0	2	2
10^1-10^2	1.1×10^1	0	0	0	0	3	3
	1.3×10^1	0	0	0	0	3	3
	1.8×10^1	0	0(1)	0	2	3	3
	5.4×10^1	0(3)	3	3	3	3	3
$>10^2$	1.1×10^2	1(3)	3	3	3	3	3
	1.3×10^1	3	3	3	3	3	3

(F) C. sporogenes ATCC 10000							
$<10^1$	0.3×10^1	0	0	0	0	0	0
	0.5×10^1	0	0	0	0	0	0
10^1-10^2	2.5×10^1	0	0	0	0(1)	0	0
	2.7×10^1	0	0	0	0	0	0
	2.9×10^1	0	0	0	0	0	0
	4.8×10^1	0	0	0(1)	0(1)	2	2
	5.6×10^1	0	0	0(1)	0(3)	3	2
	7.7×10^1	0	0	2	1	2	2
$>10^2$	1.2×10^2	0(3)	3	3	3	3	3
	2.5×10^2	1(2)	3	3	3	3	3

a: complete numbers of positive liver broth tubes after 48 h incubation period in parenthesis
b: colonieforming units per gram
c: treatment of the inoculated liver broth tubes (triple tube estimation)
80/5: heated at 80°C and holding the temperature for 5 minutes
80/0: heated at 80°C wothout holding the temperature
60/10: heated at 60°C and holding the temperature for 10 minutes
10/60: inoculation temperature 20°C, heating up to 60°C during 10 minutes continously
K: without heating, inoculation temperature 20°C ("room-temperature")
C: without heating, inoculation temperature 20°C, tubes supplemented with 0.05 ml Cedoxon^R (vitamine C), stored media are not boiled for ejecting oxygen