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Use of Hygicult-tpc® in Slaughterhouse Hygiene Control

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Rahkio, M. and H. Korkeala: Use of Hygicult-tpc® in slaughterhouse hygiene control. Acta vet. scand. 1997, 38, 331-338. – Efficacy of Hygicult-tpc® contact agar to control hygiene of slaughter equipment before work was studied in 3 slaughterhouses. Brushes from pork lines had higher bacterial counts (77 ± 114 cfu on Hygicult slide) than other pieces of equipment in pork (55 ± 101) and beef (42 ± 110) lines. Low hygienic levels were found also in aprons, knives and conveyor belts. In general, equipment hygiene varied among the slaughterhouses studied (17 ± 70, 45 ± 128, 111 ± 140) and in the comparisons of certain special equipment. Hygienic levels of the equipment were classified into 3 subclasses according to Hygicult counts. Sterilizers and saws had the lowest counts; aprons, and polishing and prescalding brushes had moderate counts. Highest counts were detected in rubber backscraping brushes and steel brushes used to clean pork carcasses and in conveyor belts. Such equipment is difficult to clean, and the bacterial count of 120 on a Hygicult slide is therefore recommended as the acceptable level after cleaning. The level of 50 on a Hygicult slide is recommended as the acceptable level for equipment, which is easy to clean. The limits recommended are based on the distribution of the Hygicult counts obtained. Hygicult-tpc® is suitable for slaughterhouse hygiene control with an incubation for 72 h at 25 °C. The flexible handle enables sampling of surfaces that are hard to access with unflexible slides and agars.

machinery; equipment; surfaces; sanitation.

Introduction

Good manufacturing practices in contemporary slaughterhouses do not only include the monitoring of carcass contamination levels, but also the hygienic control of all equipment cleaned before use (*Snijders* 1988). Some researchers are of the opinion that control of the cleaning and disinfection of equipment and surfaces is considered of greater importance than the microbiological control of the carcasses (*Mackay & Roberts* 1990). Although a visual examination can be carried out to monitor cleanliness, objective, simple and rapid microbiological methods are also needed (*Mackay & Roberts* 1990, *Huis in't Veld et al.* 1994). In order to verificate the monitoring *Untermann et al.* (1997) have recommended monthly bacteriological sampling to prove the effectiveness of cleaning and disinfection.

For surface sampling The Nordic Committee on Food Analysis (*NCFA* 1987) has recommended swabbing followed by a pour or spread plate count after dilutions, or contact agars with incubation durations of either 3 days at 20-22 °C or 2 days at 30 °C. When taking surface samples by the contact agar method *Cousin* (1982) and *Nortje et al.* (1989) have employed 30 °C for 24 h to obtain faster results.

Hygicult-tpc® is widely used in Finland (*Meri-*

virta & Uutela 1991). Efficacy of Hygicult-tpc® contact agar has previously been studied with the incubation of 37 °C for 24 h, according to the manufacturer's recommendation (*Merivirta & Uutela* 1991, *Rahkio et al.* 1992, *Napravnikova & Budig* 1994). However, a lower incubation temperature of 25 °C has usually been preferred if samples originated from meat plants or slaughterhouses (*Ingram & Roberts* 1976, *Nortje et al.* 1990). When surface samples have been taken, either certain equipment has been investigated (*Rahkio et al.* 1992) or the equipment has not been specified. We have not found any comparable studies on equipment contamination among different slaughterhouses in the literature.

In this study, contamination levels of different kinds of slaughterhouse equipment employed in pork and beef lines were evaluated using the Hygicult-tpc® method. The slides were incubated at 25 °C for different time periods. Hygienic classifications of equipment were then determinated from the levels of bacterial contamination obtained by the Hygicult-tpc® method.

Materials and methods

Samples

Bacterial contamination of slaughter equipment was determinated at 3 slaughterhouses (A, B, C). All the slaughterhouses had both pork and beef production. Slaughterhouses A and C produced 10 mil. kg and slaughterhouse B 30 mil. kg meat annually. Slaughterhouses A and C were over 30 years old and repair had been done during last decade. Slaughterhouse B was under 10 years old.

Samples were taken from equipment, tools and surfaces of an unclean and a clean part of the pork and beef lines and from equipment kept in storage rooms from 5 to 7 a.m before the workday began. The division between the unclean and clean part of the line was according to *Snijders et al.* (1984). In order to avoid any variation in the sampling technique, all samples were taken by the same individual (MR). Washing and disinfection were carried out on the previous day after work by an outside cleaning service. A foaming wash was performed daily at slaughterhouses A and B and twice weekly at slaughterhouse C. Total number of samples was 950, with 312 in slaughterhouse A, 338 in slaughterhouse B and 300 in slaughterhouse C.

Hygicult-tpc method

Samples were taken with Hygicult-tpc® contact agar slides (Orion Diagnostica, Espoo, Finland). Each slide has an area of 9.6 cm^2 of ready-to-use agar on both sides. The composition of the agar is tryptose, yeast extract, glucose, agar agar, Lecithin and Tween 80. The slides are packed separately in plastic boxes which are easy to transport for both pre- and postsampling without any contamination hazard. The slides were in contact with the sampled surface for 10 sec. The slides were incubated at 25 °C and the colonies outgrown counted after 24, 48, 72 and 96 h. According to *Niskanen & Pohja* (1977) contact agar can be accurately assessed provided the number of bacteria is below 50 cfu on one cm^2. Thus the maximum number of colonies that can be counted on Hygicult is about 500.

Statistical analysis

Statistical analyses were carried out by the two-sample Student's-test and the GLM (General Linear model) (*Statistical Analysis System* 1985). Whenever the GLM produced a statistically significant difference for a variable, Duncan's multiple range test was employed to perform simultaneous comparisons of the levels of the factor. All statistical tests on bacterial counts were performed using logarithmic (log_{10}

cm²) transformations. If the Hygicult agar failed to produce growth, the count was estimated at 0.15. When the number of colonies on a slide exceeded 500, the number was estimated at 1000. Counts shown in the Tables are the means and quartiles of the actual Hygicult culture counts to give the data a more practical application.

Results

Effect of incubation time on the number of bacteria obtained with Hygicult-tpc at 25 °C is presented in Table 1. The number of visible colonies increased according to incubation time and were significantly lower after incubation of 24 and 48 h compared to the respective counts after 72 h and 96 h (Student's t-test). However, the actual difference between 48 h and 72 h was so slight that its practical significance is little. Counts given in Tables 2-4 represent an incubation of 72 h.

Slides with bacterial overgrowth were very rare and were, therefore, considered insignificant even after the 96 h incubation. After 24 h the number of samples over 500 cfu was 4 (0.4%), after 48 h, 7 (0.7%), after 72 h, 15 (1.5%), and after 96 h, 18 (1.8%), respectively. The number of samples that failed to grow was 522 (55%) after 24 h, 306 (32%) after 48 h, 250 (26%) after 72 h, and 216 (23%) after 96 h of incubation. After 72 h the number of blank Hygicult agars was only 13 (4%) in slaughterhouse C. The high number of slides without colonies found in slaughterhouses A and B (26% and 36%) after 72 h was likely a genuine result and the cleanliness of the equipment in these 2 slaughterhouses may be said to represent a high level of hygiene.

Effect of different factors on equipment contamination was tested by the General Linear Model (GLM). Bacterial counts varied according to day of sampling ($p = 0.02$), section of the line sampled ($p = 0.01$), equipment ($p<0.0001$) and slaughterhouse ($p<0.0001$). However, Duncan's test revealed that the samples taken on different days did not differ ($p>0.05$). Bacterial contamination of equipment in the clean part of the line (32 ± 98 cfu on a Hygicult slide of 9.6 cm²) was significantly lower than in the unclean part of the line (58 ± 109 cfu) or on the aprons and knives in the store room (88 ± 163 cfu) according to Duncan's test. An interaction between slaughterhouse and equipment was found by the regression analysis, which suggested that the differences between slaughterhouses could be dependent on certain equipment.

Contamination levels of equipment in different slaughterhouses is shown in Table 2. All types of equipment were significantly more contaminated in slaughterhouse C than in slaughterhouse A, whereas only knives kept in the store room and brushes in slaughterhouse B were significantly more contaminated than in slaughterhouse A. Average contamination levels of knives kept in the store rooms (133 ± 230 cfu on a Hygicult slide of 9.6 cm²) were higher than those of the knives stored beside the working points at the lines (40 ± 86). Average contamination level of aprons kept in store rooms was 54 ± 120 cfu.

Items with the highest bacterial contamination in the pork lines were the backscraping and steel brushes used to clean carcasses after singeing and the conveyor belts for edible and non-edible offals (90 ± 146 cfu on a Hygicult slide of 9.6 cm²). The least contaminated items were sawblades (5 ± 16) and rectum enclosers (2 ± 2). Average contamination level detected in beef lines (42 ± 110) was lower than that in pork lines (55 ± 101) and the variation between the equipment was lower. Conveyor belts (71 ± 165) were the most contaminated items in beef lines and the cleanest pieces of equipment were hand tools (23 ± 58) and sawblades (21 ± 57).

Table 1. Mean Hygicult (9.6 cm^2) counts and standard deviation of equipment before work as a function of incubation time.

Number of samples	Incubation periods			
	24 h	48 h	72 h	96 h
950	$29 \pm 81_A$	$47 \pm 100_B$	$56 \pm 120_C$	$57 \pm 130_C$

$_{A-C}$ Different subcripts indicate significant difference ($p<0.05$) tested by Student's t-test between incubation time.

Table 2. Hygicult counts incubated at 25°C for 72 h of equipment in slaughtering lines before work in 3 slaughterhouses.

Equipment	Slaughterhouse					
	A		B		C	
	Number of samples	Mean ± standard deviation	Number of samples	Mean ± standard deviation	Number of samples	Mean ± standard deviation
Prescalding brushes	24	22 ± 29	NA[a]		NA	
Backscraping brushes	24	$72 \pm 136_A$	NA		24	$167 \pm 163_B$
Polishing brushes	24	$3 \pm 6_A$	NA		24	$87 \pm 48_B$
Steel brushes	NA		40	112 ± 129	NA	
Plastic brushes	NA		16	38 ± 8	NA	
Brushes together	72	$31 \pm 84_A$	56	$88 \pm 116_B$	48	$127 \pm 125_C$
Rectum tie machine	NA		8	2 ± 2	NA	
Sawblades	24	$0.3 \pm 0.4_A$	28	$1 \pm 3_A$	24	$39 \pm 67_B$
Rumping knives	22	$5 \pm 7_A$	24	$23 \pm 44_A$	24	$88 \pm 152_B$
Head washing equipment	24	$5 \pm 9_A$	24	$3 \pm 5_A$	24	$159 \pm 173_B$
Hand tools	30	$2 \pm 4_A$	24	$3 \pm 7_A$	24	$71 \pm 79_B$
Knives in line	28	$12 \pm 33_A$	28	$6 \pm 11_A$	28	$84 \pm 115_B$
Knives in room	20	$15 \pm 66_A$	28	$266 \pm 306_B$	24	$96 \pm 127_B$
Aprons	24	$59 \pm 187_A$	30	$17 \pm 42_A$	24	$98 \pm 84_B$
Conveyor belts	24	$9 \pm 8_A$	24	$16 \pm 32_A$	24	$217 \pm 211_B$
Basins	24	$8 \pm 11_A$	24	$9 \pm 12_A$	24	$157 \pm 121_B$
Sterilizers	20	$3 \pm 4_A$	40	$4 \pm 18_A$	32	$81 \pm 175_B$
Total	312	$17 \pm 70_A$	338	$45 \pm 128_B$	300	$111 \pm 140_C$

[a] NA, not available.

$_{A-C}$ Different subscripts within a row indicate significant differences ($p<0.05$) between bacterial counts.

Discussion

It is suggested that the incubation for 72 h at 25 °C is used when Hygicult-tpc contact agar is used to control the equipment contamination. However, if samples are taken in one particular slaughterhouse and results are not compared with other slaughterhouses also the incubation for 48 h is acceptable. *Nortje et al.* (1990) concluded that the incubation temperature of 25 °C for 48 h to 72 h was a fast and fairly accurate method when using the agar sausage technique to determine the population of spoilage bacteria on meat-exposed surfaces.

The brushes in pork lines were dirtier than other

Table 3. Hygicult counts incubated at 25 °C for 72 h of equipment in slaughtering lines before work classified into 3 cleanliness categories.

Cleanliness category /equipment	Number of samples	Mean ± standard deviation	First quartile	Third quartile
Clean	254	22 ± 76	0.15	7
Hand tools				
Sterilizers				
Saws				
Rectum tie machine				
Moderate clean	448	61 ±135	1	51
Polishing brushes				
Knives				
Aprons				
Rumping knives				
Head washing equipment				
Prescalding brushes				
Dirty	248	84 ±133	4	120
Backscraping brushes				
Steel brushes				
Water basins				
Conveyor belts				
Plastic brushes				
Total	950	57 ±124	0.15	45

Table 4. Scale for the Hygicult-tpc agar incubated at 25oC for 72 hours used in slaughterhouse hygiene control sampling.

Hygiene	Number of bacteria (cfu/9.6cm²)	
scale	Easy to clean equipment	Difficult to clean equipment
Clean	< 20	< 60
Moderate	20-50	60-120
Dirty	>50	>120

kinds of equipment. Brushes are very difficult to clean compared to other equipment. The foaming wash method employed in the lines studied is likely not the optimal cleaning method for brushes. Changing the brushes sufficiently often is, therefore, very important. Dehairing machines have been found to be a source of pathogen contamination, too (*Gill & Jones* 1995). Although the difference was not significant, pork line equipment was more contaminated than that of beef line, excluding saws and sterilizers. The high contamination level of pork line-equipment is probably due to the contamination from pork carcasses. When the results of saws and sterilizers were further analyzed, it was noticed that the high contamination level of saws and sterilizers in the beef line was due to slaughterhouse C. Saws and sterilizers in the beef line from slaughterhouse C were so contaminated that when all 3 slaughterhouses were analyzed together these items were more contaminated in the beef than in pork line. Highest contamination levels were also found in slaughterhouse C for other types of equipment. This can be explained because the complete foaming wash and disinfection were done only twice a week in slaughterhouse C, instead of after every working day as in slaughterhouses A and B. Aprons and knives were cleaned by the workers themselves. It appeared that slaughterhouse A workers cleaned their knives properly but not their aprons. The opposite was the case for slaughterhouse B workers. In slaughterhouse C both knives and aprons were insufficiently cleaned.

Since the results of *Ten Cate* (1963,1965) a count of 100 cfu on about 9 cm^2 has been widely employed as the limit of sufficient cleaning in slaughterhouse equipment samples (*Merivirta & Uutela* 1990, *Havas* 1995). The present results indicate that the cleanliness of slaughterhouse equipment could not be evaluated by one acceptable limit of 100. On the one hand, there are some types of equipment and tools for which the limit of 100 is far too hygienic to be attained and on the other, a higher hygienic level could be required for other types of equipment. Instead of the average limit of 100, a two-point scale would give a more practical picture of the contamination level. According to Duncan's multiple range test the bacterial results of the equipment from all 3 slaughterhouses could be classified into 3 subclasses (Table 3). Sterilizers and saws had the lowest counts. Knives, aprons and polishing and prescalding brushes had moderate counts. Highest counts were detected in rubber backscraping brushes and steel brushes for cleaning pork carcasses, water basins and conveyor belts. Based on the means and quartiles of the Hygicult counts in Table 3 a recommendation scale for evaluation of Hygicult-tpc agar contact samples of easy to clean and difficult to clean slaughterhouse equipment is shown in Table 4. For easy to clean equipment, the limit of 50 cfu/9.6 cm^2 is recommended, whereas for difficult to clean equipment with rough and uneven surfaces the limit of 120 cfu/9.6 cm^2 is recommended as the acceptable level for samples taken for hygienic control purposes using Hygicult-tpc agar.

Difficult to clean equipment mainly comprises the equipment used in the unclean part of the line, such as the brushes of the pork line, where less stricter sanitation criteria can be applied. However, surfaces such as water basins are easy to clean. Although the carcasses are unlikely to come in contact with the interior or exterior of basin surfaces, work tools do come into contact with them. Therefore, the hygienic level of 50 cfu/9.6 cm^2 should be set for them. In general, the collection of microbiological samples should not be limited to sites which are easily sampled and cleaned, as the results from such sites fail to reveal critical hazards (*Sveum et al.* 1992). This is especially important in slaughterhouse hygiene. The flexible handle of Hygicult-tpc enables access to surfaces difficult to reach with unflexible slides and agars.

Hygicult-tpc appears to be suitable for controlling slaughterhouse hygiene. Althought the incubation time can be shortened, Hygicult-tpc® is not a rapid monitoring method like ATP-method (*Siragusa et al.* 1995), but rather a method for verification.

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Sammanfattning

Användning av Hygicult-tpc® kontaktagar vid kontroll av slakterihygien.

Slakterihygienen för apparaternas och redskapens del kontrollerades i tre finländska slakterier med Hygicult-tpc® kontaktagar. De maskiner som används för bortsning av griskroppar var smutsigare (77 ± 114 cfu på Hygicult-tpc® kontaktagar) än de övriga redskapen på grislinjerna (55 ± 101) och på nötlinjerna (42 ± 110). Skyddsförkläden, knivar, samt tranportbälten för slakningens biprodukter var också smutsiga. Den genomsnittliga kontaminationsnivån i de tre slakterierna varierade avsevärt: 17 ± 70, 45 ± 128, 111 ± 140 på Hygicult-tpc® kontaktagar. Slakteriapparater och redskap delades in tre klasser enligt kontaminationsgrad. De renaste redskapen,

dsv. steriliseringsapparaterna och sågarna, hörde till den första klassen. Skyddsförkläden samt de borstningsmaskiner som används före skållningen hörde till den andra klassen, medan de borstningsmaskiner som används efter skållningen hörde till tredje klassen. De rekommenderade renlighetsnivåerna för slakteriredskap är 120 cfu på 9,6 cm^2 för sådana som är svåra att rengöra och 50 cfu för sådana som är lätta att rengöra. Hygicult-tpc® kontaktagar är lätt att använda, med en inkubationstid på 72 h vid 25 °C, och lämpar sig därför utmärkt som undersökningsmetod vid kontroll av slakterihygien.

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