PCR-DETECTION OF *STAPHYLOCOCCUS AUREUS* AND ITS ENTEROTOXINS IN RAW PORK AND UNCOOKED SMOKED HAM

Viktoria ATANASSOVA and Christian RING

School of Veterinary Medicine Hannover, Centre for Food Science, Department of Food Hygiene and Microbiology, Bischofsholer Damm 15, D-30173 Hannover, Germany

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Background: In many countries *Staphylococcus (S.). aureus* is considered to be the second or third most common pathogen causing outbreaks of food poisoning, only outnumbered by *Salmonella spp.* and in competition with *Clostridium perfringens* (TODD 1983; WIENEKE 1993). Symptoms like vomitus, abdominal pain and diarrhea usually occur two to six hours after the intake of contaminated food and are caused by staphylococcal enterotoxins (SE) (TRANTER 1990). So far nine serologically distinct SEs (SEA to SEJ) have been described, all of which are heat stable proteins with a molecular weight of 27-31 kD (BALABAN and RASOOLY 2000). Thus cases of illness might occur although no viable bacteria can be isolated from the suspected food.
Ham is described as the vehicle most frequently identified associated with staphylococcal food poisoning in literature, but other foods such as chicken, pork, beef, meat dishes or salads are also commonly implicated (BRYAN 1988). In Germany in the summer of 1988 and in 1996 several persons were ill after the consumption of uncooked smoked ham. Microbiological follow-up examinations of the surfaces of whole hams revealed a prevalence of *S. aureus* of up to 10^6/g. In 2000 nearly 300 persons fell ill after having eaten a noodle dish with ham. Here unhygienic handling of the ham by the kitchen staff and insufficient cooling of the already prepared food allowed for an contamination of the food with SEA (ANON. 2000).
Modern molecular biological techniques for the detection and differentiation of pathogens gain more and more importance in food hygiene. Methods have also been described for the identification of *S. aureus* (e. g. Johnson et al., 1991), and staphylococcal strains harbouring copies of enterotoxin genes can be rapidly detected by PCR (Martineau et al., 1998).

Objective: The aim of this study was to examine the prevalence of *Staphylococcus aureus* and its enterotoxins in fresh pork delivered by different suppliers by means of PCR and to monitor the prevalence of the pathogen in products derived from this meat.

Material and Methods: Samples: A total of 225 samples was analyzed. 75 pig hindquarters from various suppliers of fresh meat were unmistakably labelled at the beginning of the fabrication process of smoked ham. At three different stages during the fabrication process (raw pork, day 0; salted meat, day 42 and ready-for-sale uncooked smoked ham, day 64) samples were taken and examined.
Reference strains: Reference strains producing staphylococcal enterotoxins A (619/93), B (62/92), C (1299/93), D (1634/93) or no enterotoxin (8325) were kindly provided by the "National Reference Laboratory for Staphylococci", Wernigerode, Germany.

Preparation of bacterial DNA: 25 g of the samples were suspended in 225 ml of Baird-Parker broth (with 0.01 % potassium tellurite) and incubated at 37°C for 24 h. After centrifugation of 2 ml of this broth the bacterial pellet was resuspended in 150 µl TGE buffer, then 4 M guanidine thiocyanate solution and 15 µl diatomaceaous earth (SiO_2) were added. After another centrifugation step the pellet was washed several times with TGE, 70% ethanol and acetone. Finally 15 µl A. dest. were added and the sample was heated to 56°C for 1 h. After centrifugation the pellet was discarded and the supernatant was used for PCR analyses.
Detection of *S. aureus* by PCR: For the detection of *S. aureus* by means of PCR 1 µl of the solution containing the bacterial DNA was transferred to a 20 µl PCR mixture and thermal cycling was performed exactly as described by MARTINEAU et al. (1998).
Detection of staphylococcal enterotoxin genes by PCR: For the detection of the staphylococcal enterotoxin genes A to D the primers and conditions of thermal cycling described by Johnson et al. (1991) were used. 1 µl of the DNA preparation was added to a reaction mix according to POLLARD et al. (1990).
All PCR-amplified samples were analyzed by gel electrophoresis using horizontal 2 % (w/v) agarose gels.

Results: So far 135 of a total of 225 samples have been completely analysed, the last samples are currently being examined. Figure 1 shows the expected size of the amplified PCR fragments of enterotoxin genes A to D. The detection of SED genes by PCR proved to be difficult and did not always work satisfactorily. The results of the PCR analyses are presented in table1.

Discussion: So far in this study *S. aureus* was detected in 62.2 % of the raw pork (45 samples examined). This percentage is much higher than the 22.7 % positive samples described by SCHRAFT et al. (1992) for pig hindquarters. However, whereas SCHRAFT et al. took swab samples of the meat surface that were examined culturally we did not employ cultural detection but PCR analysis. The sensitivity of the cultural technique might not have been sufficient to detect very low levels of the pathogen but on the other side PCR detects pathogens at DNA level so that a positive result does not necessarily mean that the microorganisms are still viable. However, a positive PCR result for *S. aureus* shows that the pathogen, at potentially toxigenic levels, has been present in the sample. Since enterotoxins might still be contained in the food although no viable bacteria are left such samples have to be looked at critically.
In this study raw pork delivered by different suppliers showed striking divergences in the prevalence of *S. aureus*. Whereas the pathogen was detected in 75 % of the raw pork delivered by one supplier and 87.5 % of these positive samples were also shown to harbour enterotoxigenic strains of *S. aureus*, PCR gave positve results only in 57.1 % of the raw pork samples from another supplier. At a high percentage the isolates cultivated from the meat samples delivered by the same supplier revealed identical characteristics. Therefore it seems possible that the pathogen did not originate from the slaughter animals but were spread onto the carcasses during the further handling of the meat. *S. aureus* is found in 30-80 % of the human population and one to two thirds of these carriers harbour enterotoxigenic strains (ISIGIDI et al., 1992; WIENEKE et al., 1993). To ensure the impeccable quality of the product the outmost hygienic precautions have to be taken during the slaughter and further processing of the meat. Unfortunately neither specific information on the suppliers nor on the handling of the meat prior to the delivery could be obtained.

By PCR *S. aureus* genes were detectable in 57.8 % of 45 salted meat samples and in 35.6 % of 45 ready-for-sale smoked hams. In approximately a third of these positive samples staphylococcal enterotoxin genes were also found. The molecular biological detection of staphylococcal enterotoxins by PCR is faster and easier and to perform than classical culturing detection. However, for some as yet unknown reasons the detection of the SED gene proved to be difficult and did not always work satisfactorily. Results concerning this staphylococcal enterotoxin gene have therefore to be looked at critically.

Conclusion: In this study we examined the prevalence of *S. aureus* and its enterotoxins in raw pork and smoked hams produced from this meat using PCR analysis. So far *S. aureus* genes were detected in 51.9 % of all examined samples and 35.6 % of the ready-for-sale smoked hams reacted positively in the PCR. Concerning the consumers' health these results have to be looked at critically. Although the prevalence of the pathogen decreases during the production process, to minimize potential health hazards special attention should be paid to the microbiological status of the source material. Good manufacturing practice and high hygienic levels help in preventing new or re-contamination of the products with *S. aureus* during the fabrication process.

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Data:

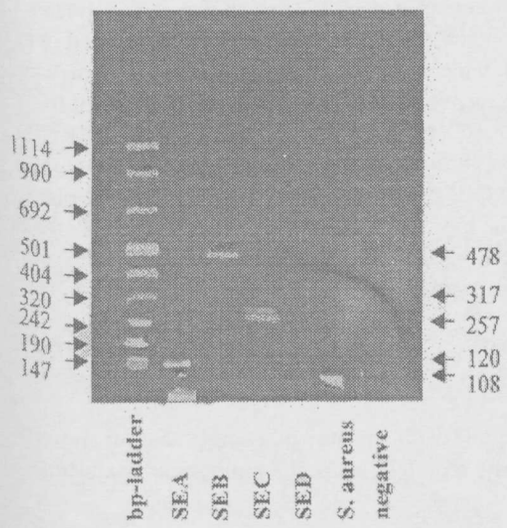

Fig. 1: Agarose gel electrophoresis of the amplified PCR fragments for the staphylococcal enterotoxin genes A to D (SEA-SED)
Primers described by MARTINEAU et al. (1998) were used for the detection of *S. aureus;* negative: negative control

Analyte	raw pork (%)	salted samples (%)	ready-for-sale smoked ham
S. aureus	62.2	57.8	35.6
SE genes[a]	57.1	57.1	37.5

[a] percent of *S. aureus* strains that had genes for SEA-SED

Tab. 1: Prevalence of *S. aureus* and SE genes