

**IDENTIFICATION OF CRITICAL POINTS IN MONITORING
THE ORIGIN OF BEEF****H.-J. Rösler, S. Jäsert, L. Döring, S. Maak**

The public increasingly demands transparency in the production of foodstuff. Beside well characterized and documented production methods the reliable identification of the origin of animal derived food at any point of the production process becomes more important. Therefore, this parameter is a central point in the implementation of process - oriented quality management systems in animal production. This is especially true for beef production due to the potential relationship between BSE and Creutzfeld -Jacob- Syndrome. The DNA analysis of the animals and foodstuff produced of them provides potentially the highest level of reliability for the tracing of origin.

Aim

Aim of this project is the identification of critical points during the production process of beef with regard to the possible loss of identification. To this end, we focussed on:

- Test of a tissue sampling system for DNA analysis under practical conditions, and
- Identification of critical points during slaughter and processing.

Material and methods

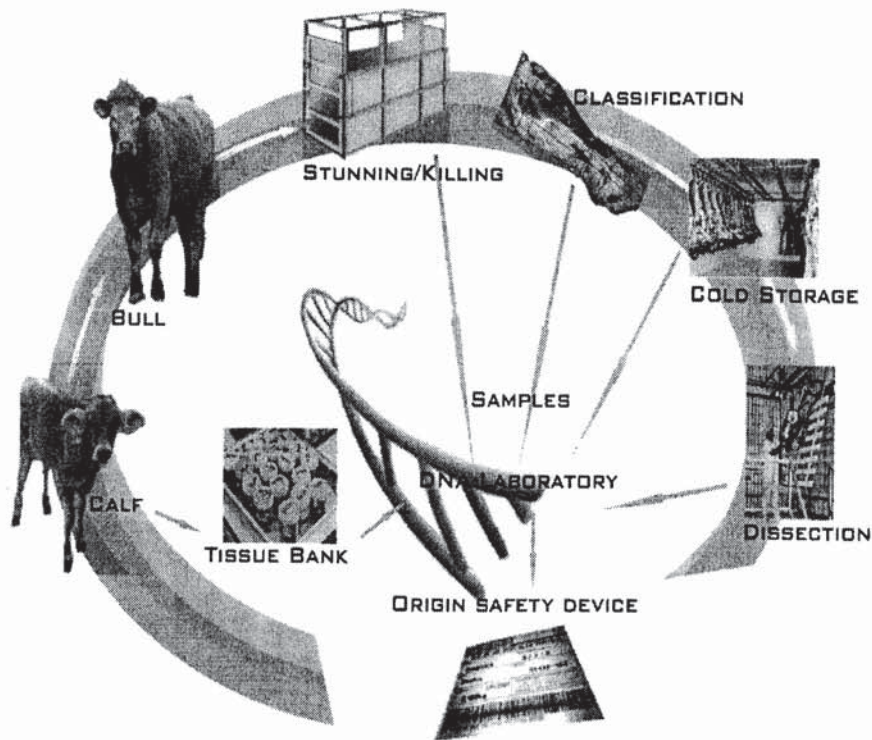
Tissue was sampled from a total of 23,000 cattle by application of the legally demanded ear tag at birth as described elsewhere (Rosier et al. 2002). The applied ear tag system allows the reliable assignment of the samples to the respective animals. The sampled tissue was registered in a data bank and

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H.-J. Rösler, S. Jäsert, L. Döring, MRA Saxony-Annalt e.V., Halle/S; S. Maak, Martin-Luther-University Halle-Wittenberg, Institut of Animal Breeding.

stored at -80 °C. Three hundred and thirty-five animals were randomly selected and monitored during slaughter and processing by staff of the MRA. The animals were slaughtered at different abattoirs and at different days. The identity of the slaughtered animals was registered from the ear tag immediately for stunning. Tissue samples were taken at:

- stunning and killing (116 samples),
- carcass classification (299 samples),
- cold-storage depot (120 samples) and
- carcass dissection (19 samples) by comparable sampling systems.



The respective tissue samples were removed from the tissue bank and comparatively analyzed with the assigned samples of the carcasses. DNA extraction was performed with the EZNA Tissue DNA Kit (peqLab, Erlangen, Germany) according to the manufacturer's guide. Microsatellite amplification was done with the Stockmark Kit for cattle (Applied Biosystems, Foster City, USA) essentially as described by the supplier. The amplified mixes are electrophoresed on an ABI 377 automatic sequencer.

Results

A total of 554 samples was taken from 335 animals. Among them, three animals (0.9%) could not be assigned due to missing samples from ear tag application (failure of the sampling system). However, for each of the both animals the samples from stunning, classification, cold-storage depot and dissection were identical. Further 8 animals were excluded from the analysis due to insufficient quality of tissue samples from slaughter and processing. A total of 4 samples indicated contamination with other samples as revealed by the occurrence of more than two alleles in some markers. All of those samples again taken at the cold-storage depot.

Totally, 43 out of 554 tissue samples (7.8 %) could not be analyzed with molecular markers. The reasons were low sample weight and sample degradation, respectively resulting in insufficient amounts and quality of DNA for subsequent marker analysis . The failure of sampling was higher during stunning and killing (13.8%) and in the cold-storage depot (14.2%) than at the other points of sampling. This problem could be resolved by modification in sampling and sample conservation.

In 313 out of 324 animals (96,6%) the identity of the samples was confirmed at all stages (ear tag sample - carcass dissection). In 11 animals contradictions were detected between analysis results from different stages (Table).

Table - ANIMALS WITH CONTRADICTORY ANALYSIS RESULTS AFTER MULTIPLE TISSUE SAMPLING

Animal	Time point of tissue sampling			
	Birth	Stunning/Killing	Classification	Cold storage
Rind 887	correct (reference)	wrong	wrong	wrong
Rind 223	correct (reference)	wrong	wrong	wrong
Rind 001	correct (reference)	-	wrong	-
Rind 245	correct (reference)	-	wrong	-
Rind 387	correct (reference)	-	wrong	-
Rind 462	correct (reference)	-	wrong	-
Rind 591	correct (reference)	-	wrong	-
Rind 677	correct (reference)	correct	wrong	wrong
Rind 004	correct (reference)	correct	correct	wrong
Rind 549	correct (reference)	-	-	wrong
Rind 929	correct (reference)	correct	wrong	correct

As expected problems with identity increase during processing. The percentages of wrong assignments were 1.72% at killing, 3.01% at classification and 4.17% at cold storage, respectively. The wrong assignment of two animals already at stunning is most probably due to reading errors from the ear tag. Alternatively, a loss of both ear tags during lifetime and a subsequent application of a wrong ear tag may be the reason, however, with much lower probability.

Carcass classification was identified as a major cause for loss of identification. At this point by far the most samples were taken (9 wrong assignments among 299 samples). The main reason for this could be the temporal removal of carcasses from the slaughter line. Four of the 9 misclassified animals were slaughtered at the same day. From one animal two samples were taken but assigned to two different ear tag samples. The remaining two samples were mixed up.

In 1 animal mis-assignment occurred exclusively during classification with correct assignment before (stunning/killing) and after this point (cold-storage). In all other cases mis-assignments occurred in all subsequent places following the first wrong assignment. There were no wrong assignments during carcass dissection. This, however, may be due to the low samples size (19 animals).

Conclusions

- A reliable tracing of the origin of beef can be implemented on the basis of DNA analyses.
- Critical points were identified for sampling of tissue at different stages during slaughter and processing. Subsequently, the sampling procedure was improved.
- Approximately 4 % of the carcasses received wrong assignments during the slaughter process in our investigation. This points to the necessity of improvements in the quality management systems of the abattoirs.
- The costs of the applied monitoring system are too high for a broad application under practical conditions at the moment.

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IDENTIFICIRANJE KRITIČNIH TOČAKA U PRAĆENJU PODRIJETLA GOVEDINE

Javnost sve više traži transparentnost u proizvodnji hrane. Osim dobro opisanih i dokumentiranih metoda proizvodnje, pouzdano identificiranje podrijetla hrane od životinja na svakom stupnju proizvodnje postaje sve važnije. Stoga je ovaj parametar središnja točka u

provođenju sustava menadžmenta procesa orijentiranog na kakvoću u proizvodnji životinja. Ovo je osobito točno za proizvodnju govedine zbog moguće veze između BSE i Creutzfeld – Jacob sindroma. DNA analiza životinja i hrane od njih proizvedene pruža potencijalno najvišu razinu pouzdanosti u traganju za podrijetlom.

Zaključci

Pouzdana traganje za podrijetlom govedine može se provesti na osnovi DNA analiza.

- Identificirane su kritične točke za uzimanje uzoraka tkiva na različitim stupnjevima klanja i prerade. Nakon toga postupak uzimanja uzoraka se poboljšao.
- Prosječno 4% polovica dobilo je pogrešne naljepnice u postupku klanja u našem istraživanju. To upućuje na potrebu poboljšanja sustava menadžmenta kakvoće u klaonicama.
- Troškovi primijenjenog sustava praćenja za sada su previsoki za široku primjenu u praksi.

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