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DNA-based traceability of pork

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Introduction

Diseases and epidemics like BSE and foot- and mouth disease have recently affected European livestock populations. Therefore, the consumer is asking increasingly for more detailed information concerning the health of the animals to ensure the safety of consumable animal-derived products. However, this information is difficult to come by because of the different stages of production an animal has to go through before its products arrive on the market.

These circumstances initiated the development of a traceability system to record, where an animal was born, fattened, and slaughtered. This new concept makes it possible to identify the origin of an infected animal in the cases of a disease or epidemic outbreak, or even for the misuse of medication or use of illegal food. The traceability systems currently in use are based on special ear-tags, subcutaneously implanted chips or labelling. In principle, these systems ensure an accurate record of the steps the animal goes through before being slaughtered, but the „cutlet“ at the end of the chain nevertheless remains anonymous. After the animal has been slaughtered, there is no chance to run down the animal's history, thereby giving the consumer full transparency.

Instead, a system based on DNA information holds more promise in this regard. For cattle, the Generatio GmbH Company generated an „Animal Trust Center-Infrastructure“ database to outline the whereabouts of individual animals. This internet database contains all necessary information of the animal, its farm of origin, and their marketing label. The origin of any meat products can be identified by typing 10 DNA markers.

In contrast to the situation in cattle, where cows have an expected useful life of 2,5 years with about 3 calves in this time, a sow produces about 40-60 piglets during her reproductive lifetime. Thus, it is prohibitively expensive and demanding to record each piglet to enable the tracing of pork by direct DNA fingerprints. Instead, we make use of the high multiplier effect in sows to ensure the efficiency of the typed parents while minimizing the costs per slaughtered pig.

The goal of the project is to map out an optimal strategy to guarantee an economical and safe DNA-based traceability in pork via the genotyping of all parents in the system and inclusion of supplementary available information.

Pilot Project

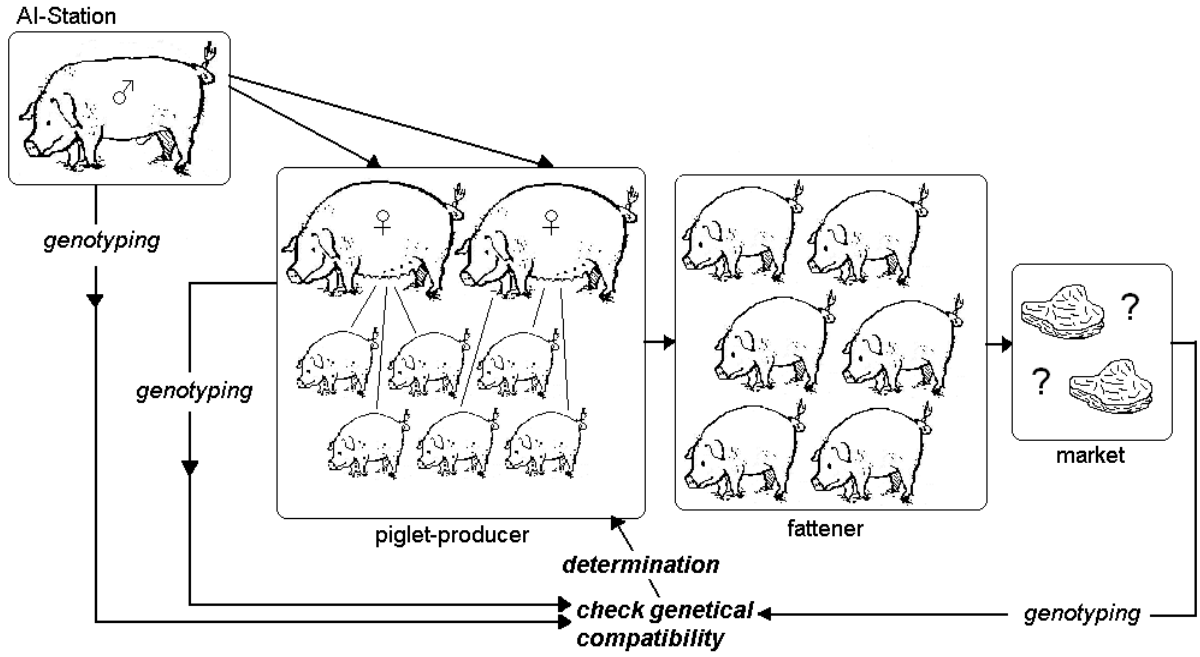
To assure the consumer a high level of accuracy in the pig sector, a pilot project was financed by the Bavarian Ministry for Environment, Health and Consumer Protection. The scientific support is provided by the Chair of Animal Breeding of the Technical University of Munich, and additional involved institutions are:

- Community of interest in certification GbR, an association of 36 farmers (piglet producers and fatteners) with the view to bringing traceable, accredited pork on the market.
- LKV Bavaria e.V., which make all required information about the animals in the system available.
- A laboratory for creating the genotypes and an IT-company for information technology.

Rationale

Because of the economic considerations mentioned above, a genotyping system where not every single piglet needs to be typed is used as a traceability method. Instead, only all operating parents in the system are sampled to assign their progeny to the true parents and by this mean to their farm of origin.

Fig. 1: Overview of genotyping system



After the extraction of DNA from hair samples of both the sows and boars, microsatellite marker loci are used to genotype them. Because these genotypes

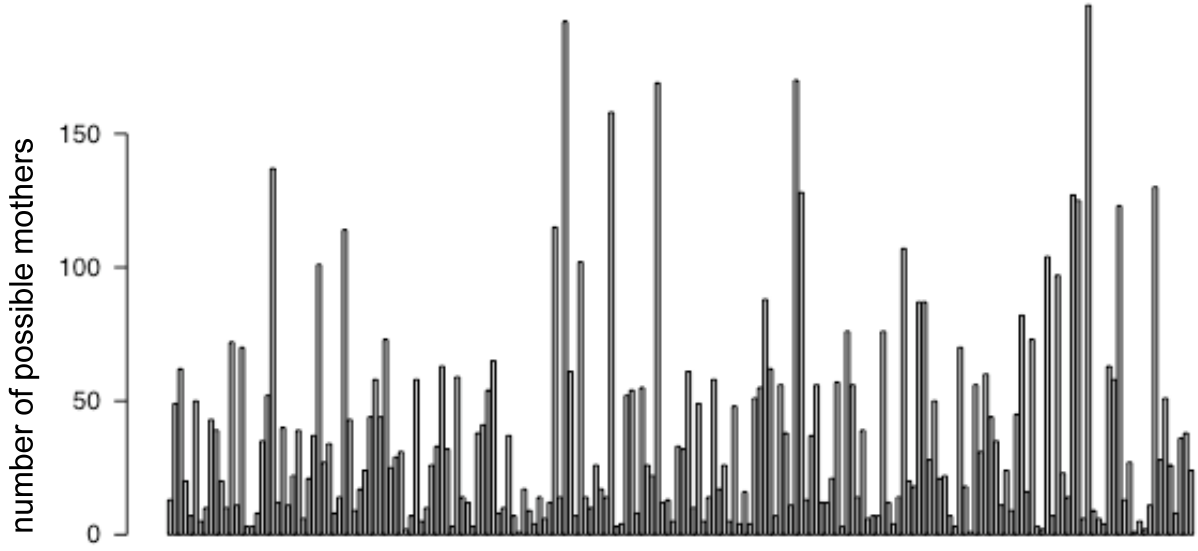
are the basis for our proposed indirect method of traceability, it is crucial that all potential parents in the entire stock, including replaced animals, are genotyped. It is only in this way that animals can be excluded as parents of a typed "cutlet" using genetic information.
Our indirect comparison is based on heredity, where each offspring receives one of the two alleles per marker locus from its mother and one from its father. Thus, in contrast to a direct genotype comparison, where the fingerprint of a sample has to be identical with a previously stored probe of the same animal for a positive match, our indirect comparison requires only one allele per marker locus of the sample to match that of the mother. As such, many potential parents can rapidly be excluded. The efficiency of exclusion, and thus also the probability to find the correct mother and father out of the animals in the whole system, increases with an increasing number of markers. Therefore, it is possible through this indirect genotyping to locate the origin of a „cutlet" from a butcher's shop by singling out the true mother and thus the farm of origin.

Approaches

The production line currently considered in the pilot project comprises about 4600 sows. With the indirect method, it is not unlikely that the genotypes of several sows are compatible with that of the probe, such that each sow can be viewed as a possible mother.
However, the following scenarios demonstrate how to improve a system based only on comparison of genotypes through the inclusion of supplemental information supplied by animal recording.

1. To identify the mother of a sample, and hence the farm of origin, using only genotype information from sows in the system requires many markers. Figure 2 shows the result of a simulation with 200 probes typed on 14 markers. Note that several were identified as possible mothers in most cases.

Fig.2: Result of traceability of 200 "cutlets" by comparison with sows only -14 Markers-



2. An additional comparison can be made by incorporating genotype information from boars used in the system, thereby excluding some sows while using the same quantity of markers to identify their offspring. The artificial insemination station sends hair samples of corresponding boars to the laboratory to type them.
3. The records of the arranged matings and produced offspring supply an additional source of information. After exclusion of the sows and boars following step 2, only animals that are known to have been mated in the requisite period are considered as parents. Mating information are compiled into the database system by the farm consultant of the LKV Mittelfranken e.V.. However, the use of these data means that the farmers have to be trusted with giving accurate mating information.

Considering the genotypes of both sows and boars and conducted matings reduces the number of markers required to correctly identify the parents. Table 1 shows the probability of exclusion from different numbers of markers with the inclusion of boar genotypes and recording of effective proceeded matings.

Tab.1: Probability of exclusion by different amount of markers
-4500 sows, 50 boars in the system-

number of markers	**simulations**	**correct parents in .... cases**
10	100	86
11	100	90
12	100	94
13	100	92
14	100	97
15	100	100

After determination of a sufficient number of markers needed to genotype pigs in the system, every typed animal will be registered in a database where all relevant information like date of birth, date of first dedication, number of litters are recorded. Software for the genotype comparison and the exclusion of the regarded matings comes into operation to find out, if a random typed “cutlet” derives from the system.

Discussion

The use of genetic fingerprints for traceability has an advantage over alternative approaches in that it allows the possibility to type „cutlets“ after slaughtering.

Thus, it provides the consumer with a high level of transparency for the pork production process.
However, to guarantee a completely safe product, the input of all farmers and producers in the system is essential. In particular, there is the need to update data continuously so that each record of an animal in the database is always complete. This demands efficient organization and discipline as well as willingness to cooperate of everyone involved.
An open question is whether an animal should be excluded as a possible parent if only one marker shows no compatibility with the genotype of the sample. For instance, spontaneous mutations in microsatellites are known, but rare, occurring in the region of 1/1000 (mutation-rate in microsatellites pig 7 x 10^{-5} per generation and gamete).
Accurate traceability based exclusively on genetic information is possible only with a very high number of markers. By including supplementary information, a reduction of this number and therefore of cost is feasible, but places increased demands on the entire system. Certainly, the accumulation of the first samples and the initial compilation of information into the database is the largest effort. Our investigations are of future interest because they can give an outlook whether this system will be profitable and applicable for larger production systems and even for the whole population. To enlight these aspects, another project with emphasis on economical benefits achievable by the proposed system is currently underway.

Conclusion

In conclusion, the DNA-based traceability in pork is possible, but the safest methods like the direct comparison or a compatibility check using only the genotypes of the sows are not cost effective. This pilot project aims to optimize the costs and information necessary to conduct a traceability concept based on indirect genotyping combined with the inclusion of supplemental information.
Currently, every potential parent in the system gets genotyped on 14 markers. First test runs showed a convincing traceability. The next step is to analyze the structure of the population more intense and to check the impact of relationship among the parents in the system on the requirements of the traceability concept.

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