Verification of correct assignment of milk samples to cows in AMS-farms by DNA-microsatellites

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Abstract

Somatic cell content in bovine milk samples enables extraction of DNA of sufficient quality and quantity which can be used for parentage verification by microsatellite genotyping.
If the DNA analysis of the milk sample excludes the sire as registered in herdbook data, there may be two possible causes.
Firstly, wrong assignment of the milk sample to the cow, secondly the registered parentage of the cow is not correct.
From 60 Bavarian farms with robotic milking systems or milking parlor ten cows of each herd were chosen during routine milk performance recording by an algorithm of the data center for milk recording. In the milk analysis laboratory the samples of the selected cows were automatically separated for subsequent DNA genotyping by GeneControl GmbH in Grub (Bavaria).
Results from DNA test were as follows:
371 samples showed correct parentage, for 19 samples incorrect paternal parentages were found (with 9 samples from a single farm as a result of poor sample handling), 25 samples were contaminated by admixture of samples from different cows and 91 samples were insufficient due to low somatic cell counts or poor sample quality.
Further tests were performed in order to evaluate the sensitivity of the DNA test concerning admixtures of milk and dependency on somatic cell count. Thanks to the high degree of automation the method could be applicable for routine verification of correct sample mapping and sample quality, but some additional effort will be needed for a better performance.

Keywords: milk sample verification, robotic milking, DNA microsatellite

Introduction

To know the correct parentage of cows under milk recording is very important for herdbook management and breeding value estimation. Additionally the correct assignment of milk samples to tested cows in milk recording has to be assured. To ensure the assignment on farms with automatic milking systems a method originally developed for genetic selection via microsatellite DNA genotyping in milk was used. The method allows confirming paternity of the cow as well as assignment of the milk sample to the cow in one pass. It also gives clues for mixed or carried-over milk.

Materials and Methods

The somatic cell content in milk permits to acquire the necessary DNA for microsatellite genotyping, although the quality is not good enough for genotyping using SNP technology.

Former research in DNA genotyping aimed for automatic and cost-efficient acquisition of DNA from samples for milk recording with the intention to use the DNA for marker based selection via microsatellites (Buitkamp and Götz, 2004 and Medugorac et al., 2004). The development of SNP technology made this method irrelevant (Krappmann et al., 2012); however microsatellite DNA genotyping is still used in parental verification today.

Using this knowledge a concept for the setup of an automatic method in milk recording was developed to randomly check the paternity of milk cows and in addition to confirm the correct assignment of the sample to the cow. This ensures the quality of milk recording. Additionally the method permits to identify milk from different cows in one sample due to equipment carry-over or deliberate manipulation.

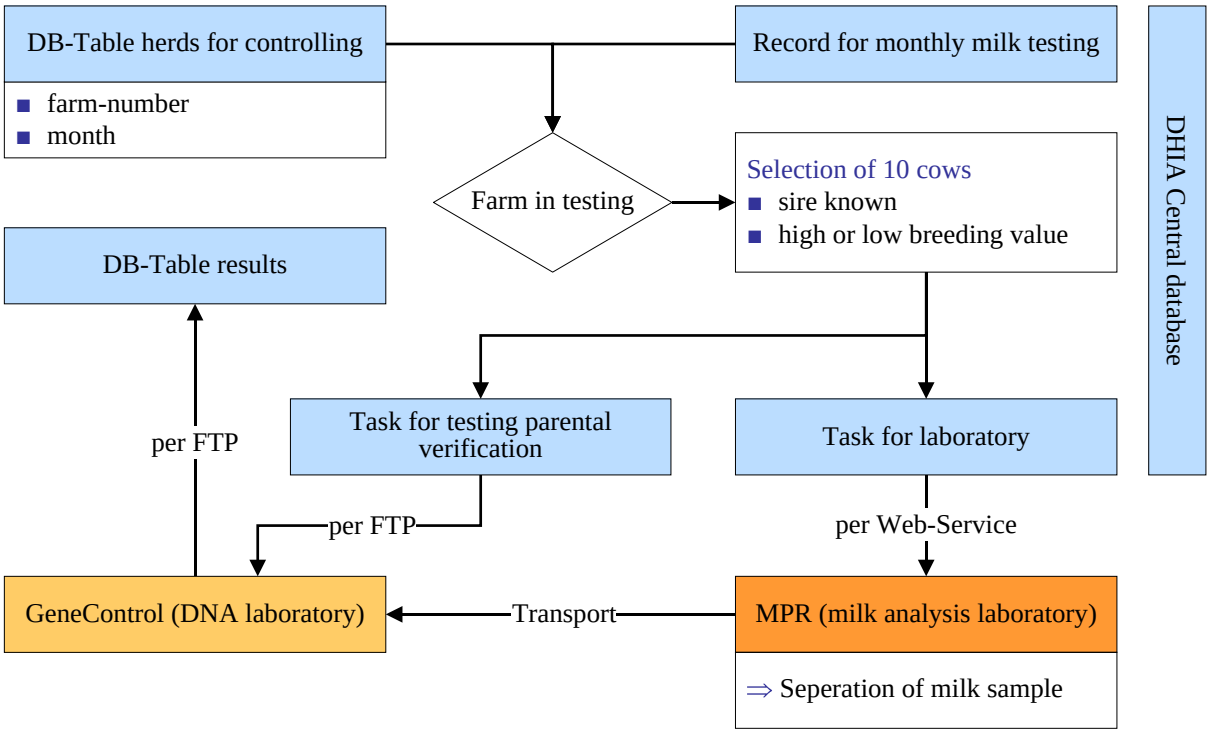

Figure 1. Method for random check of paternity and correct sample assignment.

Figure 1 shows the process sequence. It is based on the data network between dairy herd improvement association (DHIA) LKV Bayern, milk analysis laboratory MPR Bayern and DNA laboratory GeneControl GmbH in Grub (Bavaria).

As soon as the monthly milk performance recording which was assigned for additional testing has been registered in the DHIA-database, a program selects at most 10 sample bottles based on high and low milk breeding values as well as registered paternity. The selected sample bottles are automatically flagged in the milk analysis laboratory for genotyping.

At the same time the cows from which the selected samples were collected are registered online for parental verification at GeneControl. The process is similar to the one routinely used for the online-method for official parental verification by microsatellite DNA genotyping. The results are also transmitted electronically.

The possible results are:

- Father cannot be excluded
- Not the correct father
- No evaluation possible due to:
  Low DNA quality or sample contamination

Independently from the results of parental verification there are three possible levels of sample quality:

- Light indication of contamination
- Strong indication of contamination
- DNA-quality too low

60 farms, most of them equipped with automated milking systems (AMS), were selected in 2013 to check the assignment of milk recording samples to the cows using shuttles in combination with the available control lists. Due to procedural reasons 8 farms could not be checked.

Results

506 samples from 52 farms, including 2 samples from a preliminary test, were sent for DNA genotyping to GeneControl. The farms had the following milking systems:

40 farms with AMS
7 farms with herringbone milking parlor
2 farms with side-by-side milking parlor
3 farms with tandem milking parlor

Table 1. Results of DNA-resource for paternity control from milk sample.

test-result	number	%	% of usable samples	average cell count
sire wrong	19	3.8	4.9	160
sire correct	371	73.3	95.1	209
sample unusable	116	22.9		51
because of …				
poor DNA quality	91			
contamination	25			

Table 1 shows the results of DNA genotyping. The ratio of samples not suited for parental verification was above average with 22.9%, mostly because of low DNA quality. The somatic cell count for these samples strongly indicates low concentration of cells as reason for insufficient DNA yields. A smaller part of unsuitable samples was caused by contamination.
In suitable samples paternity could be confirmed with a high ratio of 95.1%. In samples where paternity was doubtful (4.9% in the present study) there are two possible causes. Either swapped samples are responsible for the negative result or the real paternity is not in accordance with the registered paternity in the herdbook. 8 of these 19 doubtful samples came from one farm, which indicates that for this special farm samples were not correctly assigned by the AMS assignment method.

Table 2. Quality of milk samples.

quality	number	% of all samples in milking parlor	% of all samples in AMS
poor DNA quality	91	23.0	16.3
strong contamination	83	11.5	17.6
light contamination	37	4.9	8.0

In contrast contaminated samples occurred more often as shown in table 2. 83 samples showed strong and 37 samples light contamination. The percentage of contaminated samples

is significantly higher on farms with AMS compared to farms with conventional milking systems. However, there is no doubt that contamination of samples occurs quite frequently independent of the milking system.

An additional experiment was conducted to closer investigate the sensitivity of DNA genotyping for carry-over by sampling or analysis equipment. Therefore milk samples were acquired from 3 cows of the Bavarian research farm in Grub with low, medium and high somatic cell count. From the 3 samples additional mixed samples were prepared in the GeneControl laboratory using 10% steps (high-medium, high-low, medium-low). The 30 thus prepared samples were analyzed by DNA genotyping. The impact of the mixing ratio on the screening of alleles of a certain marker is shown exemplarily in figure 2 and 3. Milk samples with cell counts of 797.000 (line 2) and 1.029.000 (line 1) are genotyped individually and as mixtures in ratios of 1:9, 2:8, 3:7, 4:6, 5:5, 6:4, 7:3, 8:2 and 9:1 (lines 3 to 11).

The results can be characterized as follows:

- Markers with alleles showing strong signals in screening already respond at low mixing ratios (i.e. below 10%).
- The same holds true for samples with high somatic cell counts.
- In cases of contamination caused by milk samples with low somatic cell count (below 100,000) however, only contaminations starting with a mixing ratio of at least 30% are detectable.

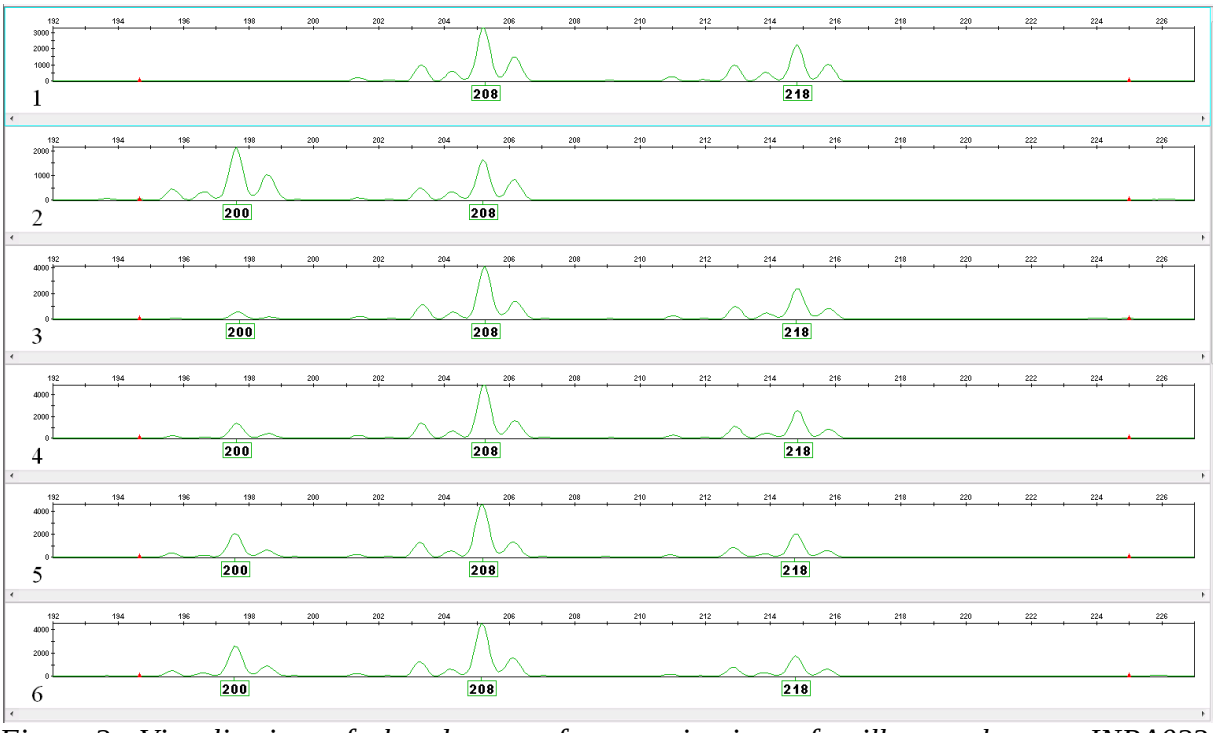

Figure 2. Visualization of the degree of contamination of milk samples on INRA023 microsatellite pattern. (part 1)

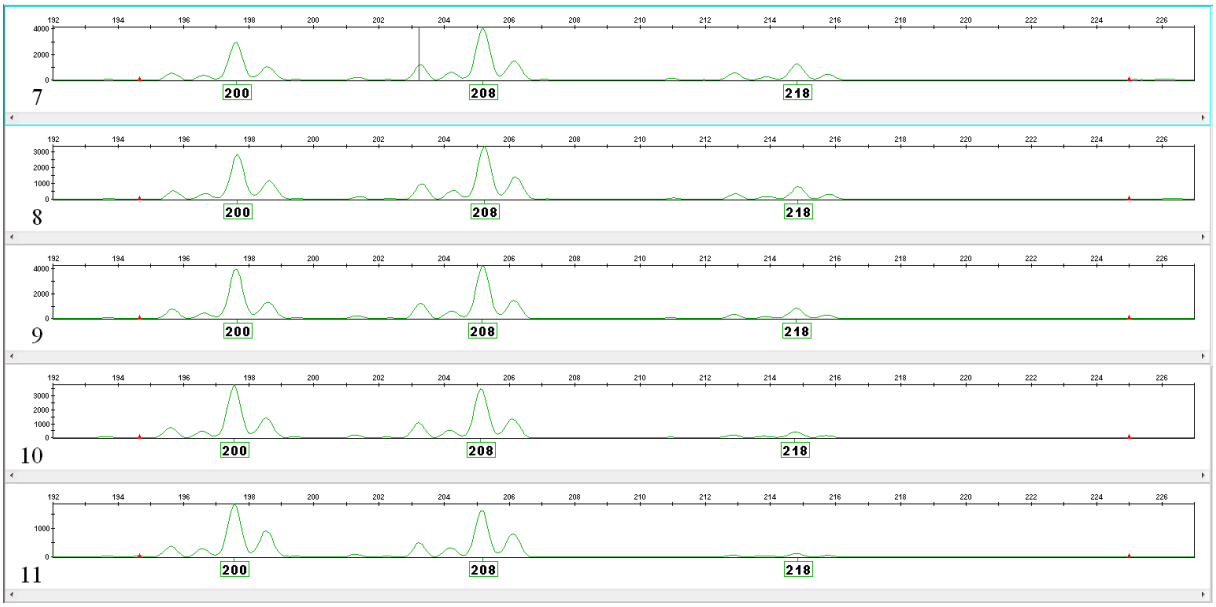

Figure 3. Visualization of the degree of contamination of milk samples on INRA023 microsatellite pattern. (part 2)

Conclusions

Analysis of milk recording samples via microsatellite DNA genotyping is a cost-efficient method due to high automation. It provides an economic way to check sample quality in milk recording and for parental verification in herdbook management. It also permits detection of contamination by carry-over or deliberate manipulation.
There are no significant costs in addition to the analysis costs for DNA genotyping, because no additional effort for sample organization is necessary. Furthermore, the method is mainly anonymized, which excludes possibilities for influencing.
Only milk samples with not too low somatic cell count should be selected for DNA genotyping because of the necessary DNA quality.

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