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

ICAR Conference
21. May 2014, Berlin

Jürgen Duda, LKV Bayern e.V., München &
Ingolf Ruß, Tierzuchtforschung e.V., Grub

Introduction

1. Correct parentage of cows under milk recording important for:
   - Herd book management
   - Breeding value estimation
   - Management of genetic defects, prevention of inbreeding
2. Correct assignment of milk samples to cows under milk recording is also important for:
   - Correct breeding value estimation
   - Prevention of manipulation
   - additional information about health status of the cow by infrared analysis

Distribution of farms in milk recording in Bavaria according to milking systems

Level at 30.09.2013

farms with	number
bucket milking	1.019
pipeline milking systems	11.481
milking parlour	11.404
AMS	1.079

Background

Verification of correct sample acquistion and sample assignment for automatic milking systems (AMS) has to be possible with low effort.

Idea: Automatic selection of milk samples from the routinely monthly milk recording to check paternity with help of DNA-microsatellites

Background

Motivated by two field tests, carried out 2002 in Bavaria

Objective at the time:
Evaluation of an automatic procedure to gain a DNA-source for genotyping cows under milk recording

- Međugorač et al. (2004)
- Buitkamp (2004)

Method

- Assignment of milk samples
  from selected cows under milk recording
  according to criteria of the breeding program
- Selection of milk samples
  in milk analysis laboratory to gain DNA
- DNA-material from milk samples
  as part of somatic cell count

Results

- DNA-material sufficient for evaluation with DNA-microsatellites
- Not sufficient for SNP-genotyping (amount of high quality DNA-material too low)
- Interference by contamination

yet suited for

- Check of paternity
- Correct sample assignment
- Discovery of mixed samples

Pilot

2013

Start of a pilot to establish a highly automated procedure for verification of sample quality on farms with AMS

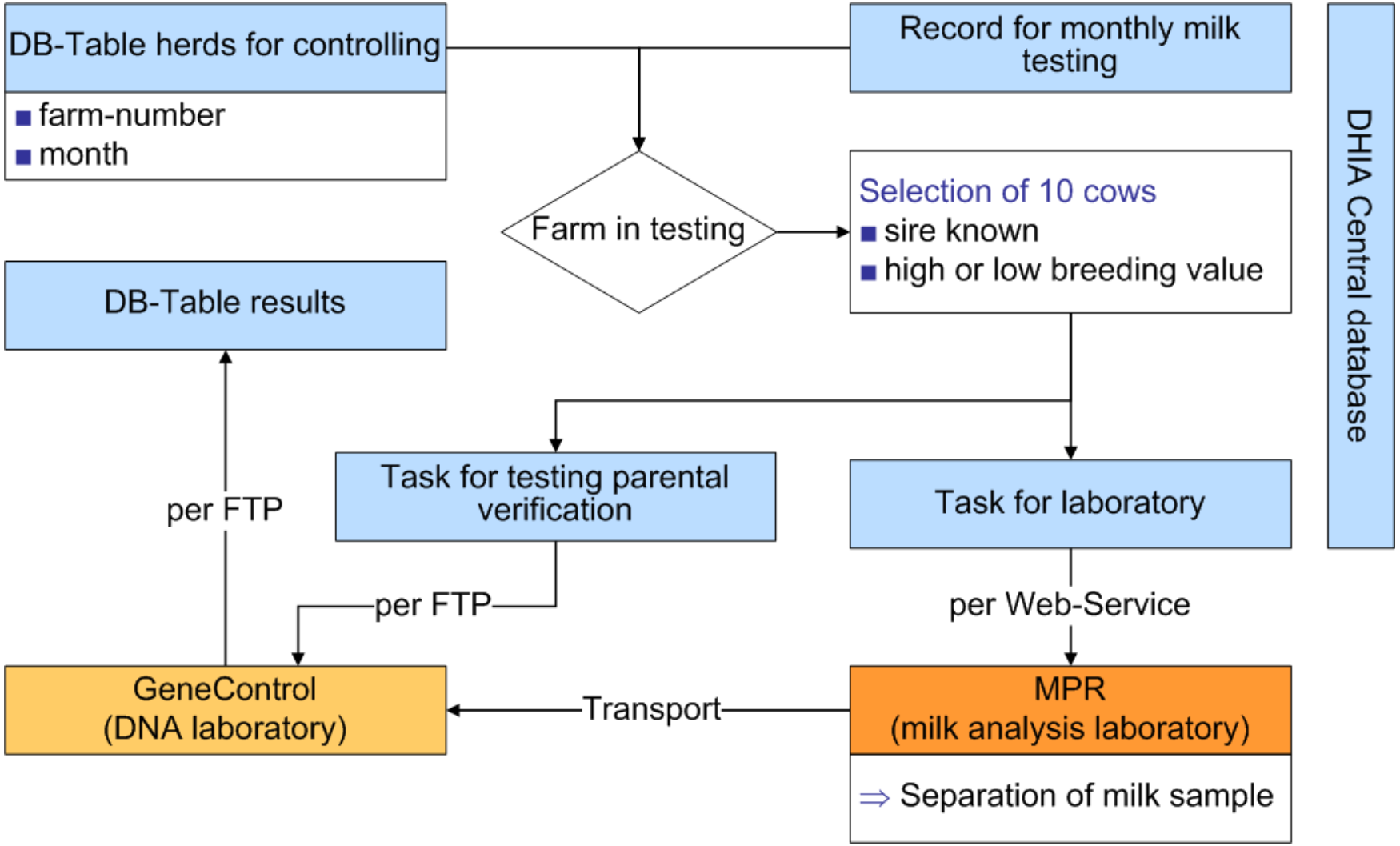
Planned process

1. Selection of 60 farms with AMS
   or automated measurement of milk yield
   in milking parlour
2. Selection of 10 samples per farm
   via a program from DHIA-database
   for additional DNA analysis
   using microsatellites

Possible results

- Sample correct (≡ father cannot be excluded)
- Sample in doubt (≡ father wrong)
  - ⇒ 2 possible errors
    - Swapped samples
    - Wrong paternity of cow
  - ⇒ Official paternity verification with DNA-analysis necessary
- Sample quality
  - Light contamination
  - Strong contamination

Process flow for automatical check of paternity and correct sample assignment



Sample bottle

Sample identifcation
via barcode number

Registering for farms

1. RDV – Berichtigungs-Dienst

Registering for farms

2. Farms
   - German Simmental, German Brown
   - Mostly artificial insemination
3. Selection of cows
   - Best 5 and worst 5 cows
     according to breeding value for total merit
   - Sires registered with DNA-profile
   - No cows from twin-births

Results

- 8 farms registered too late for automatic selection
- 52 farms evaluated with 506 samples
- Implementation in august and september 2013

  Split according to milking system
  - 40 farms with AMS
  - 7 heringbone milking parlour
  - 2 syde-by-syde milking parlour
  - 3 tandem milking parlour

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			

Results differentiated for maternal parentage and farms

result	number	%
mother o.k.	30	91
poor DNA quality	3	9
mother not o.k.	—	—

1 farm with high degree of wrong paternity
(8 samples wrong)

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

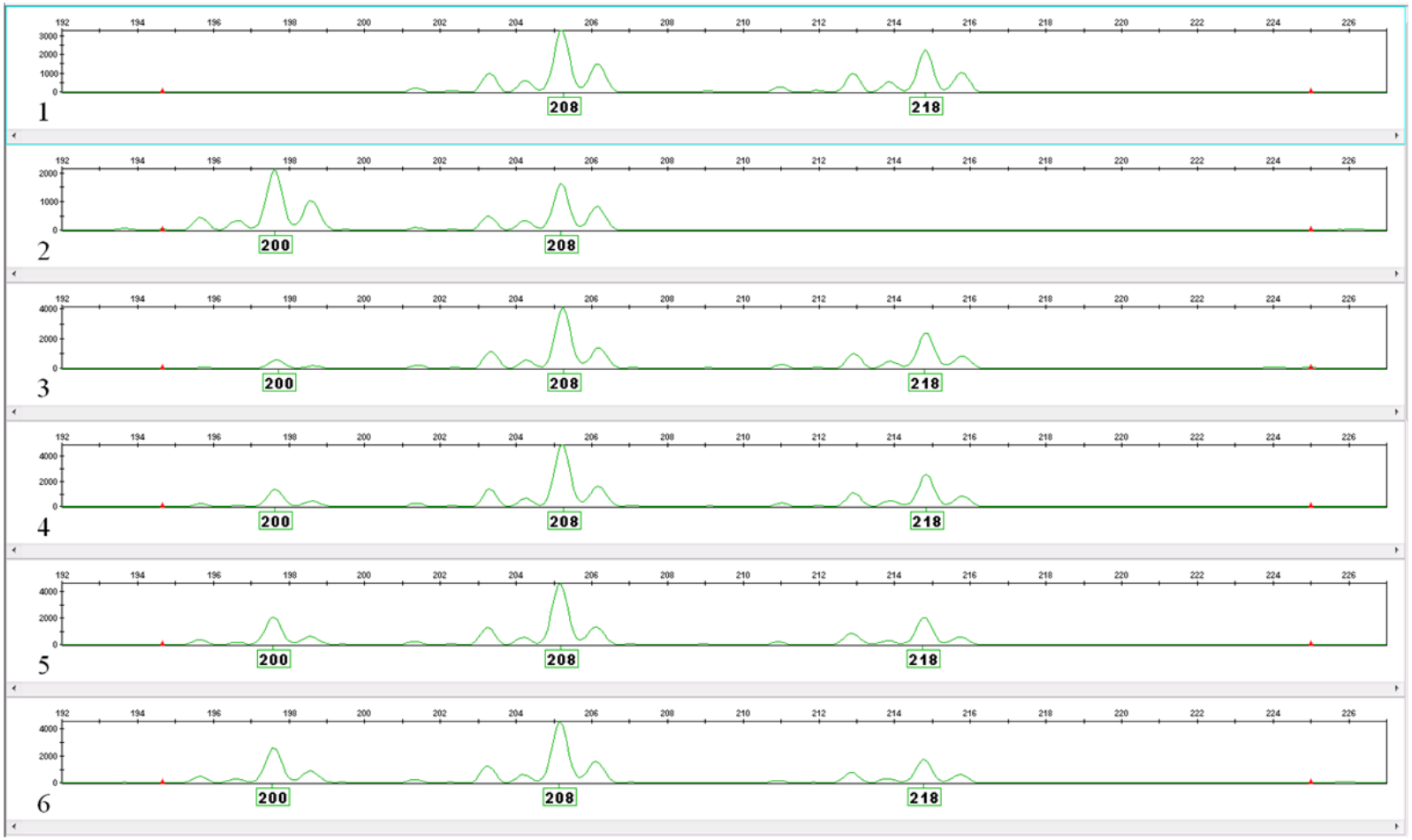
Test of sensitivity of DNA genotyping for carry-over

Selected 3 cows with

cow	somatic cell count	
1	low	(<100.000)
2	medium	
3	high	(>1.000.000)

1 sample from each cow and for 9 mixtures
in ratios at 1:9, 2:8, 3:7, 4:6, 5:5, 6:4, 7:3, 8:2 and 9:1
for each combination

Visualization of the degree of contamination of milk samples



Visualization of the degree of contamination of milk samples

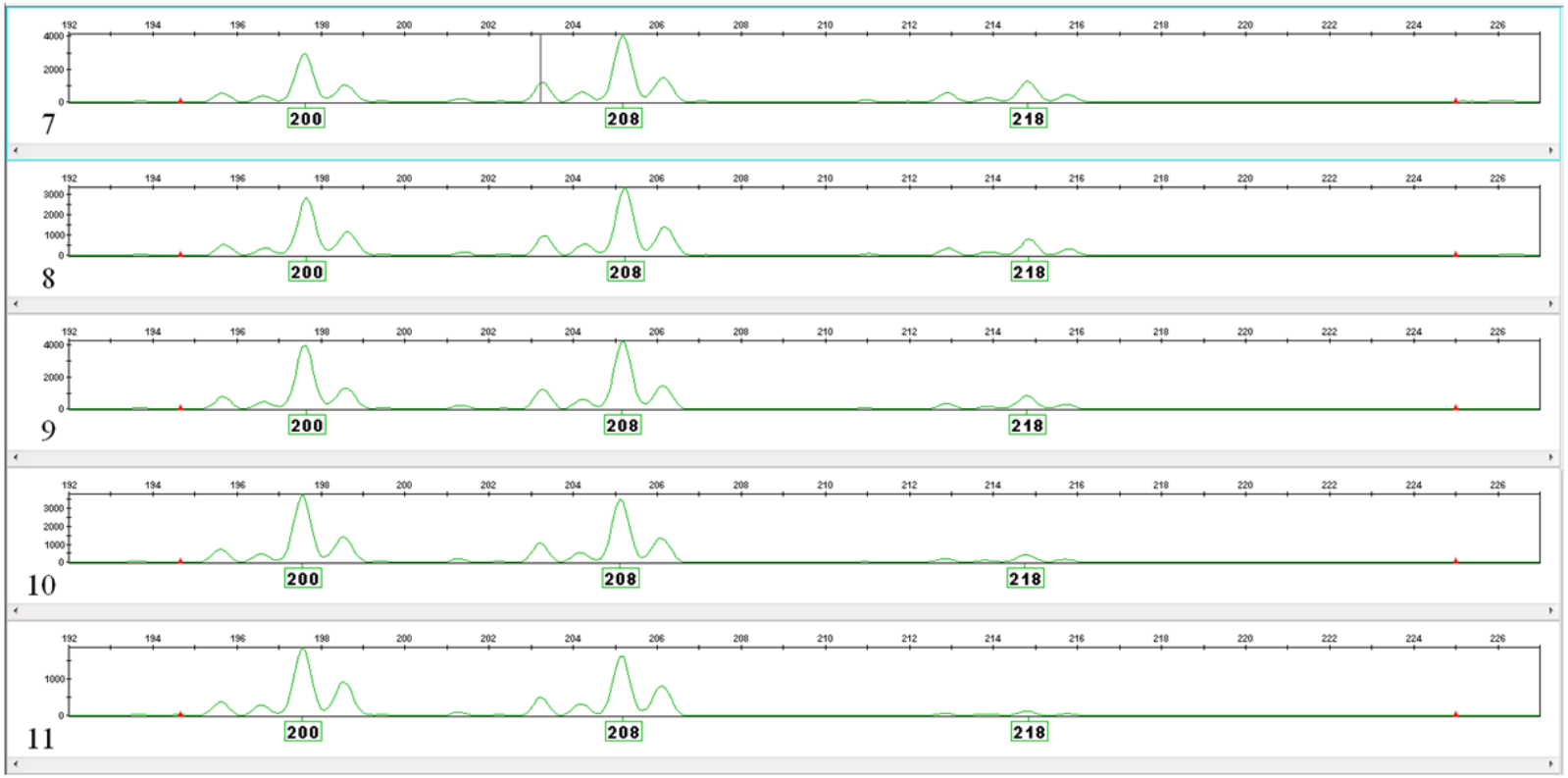
5:5

6:4

7:3

8:2

9:1



Results from the test to sensitivity

- Markers with alleles showing strong signals in screening respond even at low mixing ratios (<10%)
- High somatic cell counts cause responses even at low milking ratios
- Samples with low somatic cell counts (<100.000) contaminations are only detectable at higher mixing ratios (>30%)

Conclusion

- Analysis of milk recording samples via microsatellite DNA-genotyping is a cost efficient method because of high automation.
  No additional effort for sample organization is necessary.
- It is an efficient check for sample quality in milk recording and for parental verification in herdbook management.
- It permits detection of contamination by carry over or deliberate manipulation.

Further Steps

- In summer this year 60 other farms will be analyzed via microsatellite DNA-genotyping
- For each farm 20 samples will be automatically selected. The Laboratory of GeneControl gets additional information of cell count in order to exclude samples with low cell count.
- Farms with sampling test via LactoCorder are included in order to get a comparison to farms with own sampling systems for carry over.

Thank you very much