SESSION 43, Contact: gianluca.fiore@jrc.it

EID and DNA TRACEABILITY of ANIMALS and FOOD

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INTRODUCTION:

The past animal disease crises, such as bovine encephalopathy and foot and mouth disease, have demonstrated that a reliable traceability system is needed in order to ensure food safety and proper control of animal health. Recently, electronic identification (EID) by radio frequency has become a binding standard in EU legislation for small ruminants and horses and DNA based techniques are becoming more promising tools in the traceability of the origin of animal products. International Society for Animal Genetics and the Food and Agriculture Organization have proposed sets of DNA microsatellites (STR) as individual identification markers for the study of animal genetic diversity and for conservation purposes in different species.

Main objective of this study is to develop an EID/DNA integrated system, in fact, in order to explore the possibility to move from the present official ID system (where animals are identified according to a progressive numbering approach in a defined administrative area) to a numbering system where the EID code itself exactly reflects the individual DNA configuration.

Whatman® FTA® card

Hamilton® robotic workstation

MATERIALS and METHODS:

Two different cattle groups were targeted in terms of genetic distance. 100 dairy cattle from a single farm were used as closely related group and 100 fattening cattle were studied as a group has assumable genetic distances between members. Blood, ear-cut biopsies and nasal swab samples were collected from 200 cattle (600 samples in total). Whatman® Indicating FTA® Cards (WB120306) were used for sampling of blood and nasal swabs. Additionally, blood samples of 100 dairy cattle were also sampled into BD-vacutainer® tubes (367864) and stored at -20 ºC for future reference. 100 meat samples from slaughtered cattle and 96 milk samples from dairy cattle (4 cattle were out of lactation period) were collected. 2 x four groups of mixed samples were prepared from both meat and milk samples. All animal products and mixtures were stored at -20 ºC. Genomic DNA was extracted from ear-cut biopsies, meat and milk samples by using the Maxwell 16 tissue DNA purification kit (Promega), DNA purification of blood and swab samples (FTA® cards) was automated on the robotic workstation MLSTARlet (Hamilton®) according to the manufacturer's instructions. Amplification of eleven STR markers (TGLA227, BM2113, TGLA53, ETH10, SPS115, TGLA126, TGLA122, INRA23, ETH3, ETH225, BM1824) was carried on according to the kit protocol conditions in a thermal cycler (GeneAmp PCR System 9700 Applied Biosystems) using StockMarks® for Cattle Bovine Genotyping Kit by Applied Biosystems. PCR products were analyzed on 3130 XL Genetic Analyzer (Applied Biosystems) and results were evaluated by Genemapper software v. 4.0 (Applied Biosystems). A project database was created on Office Access, MS® Office Professional Edition 2003 for data entries and analyzing the results and has functioned as national database in the research. A Windows® compatible software (CSA, version 2) was developed to convert the analyze results of STR alleles into an alphanumeric EID code **(DEID)** with a bidirectional compression algorithm, in order to convert STR configuration in an EID code to be applied to the live animal and to trace back the ID allocated code from the STR sequence.

RESULTS:

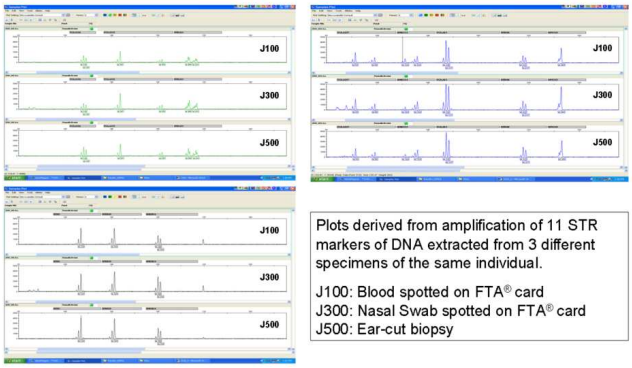
In dairy cattle group, 31 animals were sisters from 14 different mothers (2 or 3 sisters per mother) in the same farm, and 99 out of 100 cattle were Italian friesian and 1 was Bruna alpina. Fattening cattle were from 58 different holdings, belonging to 8 different breeds (Marchigiana, Charolais, Limousine, Friesian, Bruna alpina, Blonde d'aquitaine / Garonnese, Pezzata rossa and several crossbreds) and slaughtered in 2 different abattoirs. STR sequences of all individual samples and DEID codes of 200 animals were recorded to the database together with the conventional ID and basic pedigree information (breed, birth date, mother ID, holdings national ID) of animals.

Analyzed STR sequences of individuals (maximum 66 digits per animal for 11 STR loci) were converted to DEID codes by using CSA software. Concordances between DEID codes of animals and meat and milk products were demonstrated on the database successfully. In the reverse phase of the conversion with CSA software, STR sequences were successfully recovered from DEID codes (decoding) with 100% accuracy for all ear-cut biopsies, blood, nasal-swab, meat and milk samples of respected animals. Decoding of DEIDs was also validated in the database. Therefore, it has been proved that DEID code did not only allow the unique identification of the live animals by reflecting the STR configuration, but also – in case of testing of animal product(s) - provided the safe trace back the individual animal(s) as DNA and EID are strictly linked together by the bidirectional compression algorithm.

There were no deviations between STR sequences of blood, nasal-swabs and ear-cut biopsies of each individual animal. These samples have showed 100 % conformity for all individual animals involved in database analysis.

Further analysis are currently in progress on mixed milk and meat samples to evaluate the potential use of STR DNA genotyping technology to identify individuals in animal product mixtures. Results will be presented after the whole set of information will be fully processed

Twins were seen in both cattle groups, 1 pair in each. Twins in slaughtered cattle group have shown identical repeats in all STR alleles and other twins (in dairy cattle group) have shown variations in same alleles.



Plots derived from amplification of 11 STR markers of DNA extracted from 3 different specimens of the same individual.

J100: Blood spotted on FTA® card
J300: Nasal Swab spotted on FTA® card
J500: Ear-cut biopsy

CONCLUSIONS:

The major preliminary conclusions which can be drawn from the study are:

- According to our best knowledge, this the first study on integrating the genetic sequence into an EID code. DEID, which was converted from individual STR allele sequences of a food-animal can be a tool for animal identification as well as for food traceability measures. It will be very easy, in fact, to trace-back the animal ID information using the STR allele sequences of the food – genuine expression of the animal ID.
- FTA® cards could be used as powerful tool in collecting DNA samples, because they are easy to use and it is possible to store the cards many years in room temperature. The satisfactory genetic results obtained from nasal-swab spots on FTA® cards and ear-cut biopsies suggest that these specimens would be used in any field conditions where blood samples could not been collected.
- Genetic profiles of two animals in the group of slaughtered cattle were identical for all alleles in analyzed STR loci. After controlling their pedigree information in the research database, it was seen that they were twins. Since their genetic profiles are identical, it was concluded that they were monozygotic (MZ) twins. When reviewed the pedigree information (especially mother and birth date) of animals in the research database, another twins detected in dairy cattle group. Oppositely, their STR profiles were different and concluded as they were different animals in terms of individual genetic profiling; thus, it was concluded that they were Dizygotic (DZ) twins. It has been previously reported that MZ twinning in a cattle population for all births could vary from 0.13% to 0.74%. According to the information given above, the possibility to have MZ twins in any cattle population could limit the proposed technique and additional measures should be taken into account while discriminating animals individually.

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