P-07-24

Mobile suitcase lab for rapid molecular identification for meat adulteration (#441)

Jonas Kissenkötter[1], Susanne Böhlken-Fascher[1], Matthew S. Forrest[2], Olaf Piepenburg[2], Claus-Peter Czerny[1], Ahmed Abd El Wahed[1]

[1] Georg-August-Universität Göttingen, Department of Animal Science, Division of Microbiology an Animal Hygiene, Göttingen, Germany; [2] TwistDX Limited, Cambrige, UK

Introduction

Identification of animal species in meat products is crucial to prevent adulteration and unnecessary takeover during processing. Real-time PCR is the method of choice in performing meat quality control, however, this can only be performed at highly equipped laboratories. There is a need to a simple on-site rapid detection method. Therefore, in this study, four rapid molecular assays based on recombinase polymerase amplification (RPA) for the detection of pork, horse, chicken and turkey DNA in meat products were established.

Recombinase polymerase amplification is a promising technology to implement molecular diagnostic at the point of need outside a diagnostic facility. This isothermal and enzymatic depending DNA amplification method is carried out between 39 and 42 °C and results are available within 10 minutes. For DNA amplification, the recombinase binds to the target specific primers (length from 30 to 35 base pairs) and this complex invades the DNA double strand at the complementary site. The reaction is stabilized with single strand binding proteins, which tie up the opposite DNA strand to avoid any interference with the extension by the strand displacing DNA polymerase. For real-time detection, an exo-probe is employed, which is sliced by an exonuclease upon binding to analogue sequence leading to a fluorescence signal, which can be measured by a portable device.

For the DNA detection in meat and its products porcine, chicken and turkey mitochondrial ND2 and equine ATP 6-8 genes were selected as target genes.

Methods

In total, 20 pork and each nine horse, chicken and turkey primer combination were tested for their sensitivity. As amplicon a DNA standard of 300 base pairs for all target genes were synthesized by GENEART AG (Regensburg, Germany) and used with a concentration of 10^5DNA Molecules/µl for the primer testing. The best primer combinations of each assay were tested for their analytical sensitivity with a tenfold dilution range of the corresponding DNA Standard (10^2 – 10^0DNA molecules/µl). This was repeated eight times for each assay and by applying a probit regression analysis using STATISTICA software (StatSoft, Hamburg, Germany) the limit of detection in 95 % of the cases was calculated.

The specificity of the four assays was verified with Genomic DNA of pork, horse, cattle, chicken, donkey, turkey, duck, sheep, goat and rabbit purchased from Eurofins GeneScan Technologies GmbH (Freiburg, Germany) and vacuum-packed meat of eight animals (pork, cattle, chicken, turkey, duck, horse, lamb and rabbit), which was obtained form a supermarket chain.

In the next step, the pork and horse RPA assays were used to examine the influence of the red colour of meat juice and background DNA of other (animal-) source on the assays performances. In addition, these two assays were validated by screening fresh meat mixtures. Beef fresh meat was mixed with 10, 5, 1, 0.5 and 0.1 % of meat from the other animal sources. All procedure was conducted in a solar powered mobile suitcase laboratory.

Results

The horse, chicken, pork and turkey RPA assays detect one, two, 16 and 30 DNA molecules/µl, respectively. The assays speed was between six to eleven minutes. No cross-reaction with other species was observed. The RPA assay performance was not influenced by the myoglobin in the meat juice, while a one log decrease in the assay sensitivity was recognized due to the presence of high background-DNA. The pork and horse assays detect down to 0.1 % contamination of their target DNA in meat mixtures.

Conclusion

Four highly sensitive and specific RPA assays for the detection of pork, horse, chicken and turkey DNA were developed. The simplicity of the RPA technology makes the RPA assay very suitable for a mobile detection on-site. Thus, control of adulteration in meat and their products could be taking place directly during the production process or at the supermarket.

Notes