thermoscientific

Food Fraud prevention: The use of specific animal detection methods to minimize food adulteration

Greta Carmona-Antoñanzas[1], Jennifer Valero-Garcia[1], Amanda Manolis[2], Yolanda Pérez-Estarelles[1], Marta Izquierdo-García[1], Merche Bermejo-Villodre[1], Carlos Ruíz-Lafora[1]

[1]Instituto de Medicina Genómica, Calle Agustín Escardino 9, Parc Científic de la Universitat de València, Spain. [2]Thermo Fisher Scientific, Microbiology, Basingstoke, RG24 8PW, UK

ABSTRACT

Over the years, the food industry and authorities have developed food safety management systems to improve the resilience of supply chains to food fraud, mostly directed to prevent the fraud opportunity. While it is not the intention of food fraud to harm consumers, such act might cause distrust and even illness. This was the case in 2013 when EU authorities revealed the presence of uncontrolled horse meat burgers that were supposed to contain 100% beef. Generally, food fraud does not impose a health hazard, but in some ways they are more dangerous because the raw materials and quality control actions are unknown and untraceable.

Thus, addressing fraud should focus on being proactive in prevention and detection. Raw material monitoring should be performed using appropriate analytical methods for the verification of authenticity. Our laboratory analysed a total of 173 beef products to discover that a significant proportion of them had been adulterated with water buffalo meat, *Bubalus bubalis*. Once the adulteration event had been characterised, prevention measures were taken and a surveillance plan was effectively set up. Following the fraud detection event, beef products are routinely analysed for buffalo and results show absence of unexpected ingredients.

The present study highlights the effectiveness of implementing analytical surveillance to ensure the authenticity of food by minimising vulnerability to fraud and mitigating the consequences of food fraud.

INTRODUCTION

Over the years, the food industry and authorities have developed food safety management systems to improve the resilience of supply chains to food fraud, mostly directed to prevent the fraud opportunity. While it is not the intention of food fraud to harm consumers, such act might cause distrust and even illness. This was the case in 2013 when EU authorities revealed the presence of uncontrolled horse meat burgers that were supposed to contain 100% beef. Generally, food fraud does not impose a health hazard, but in some ways they are more dangerous because the raw materials and quality control actions are unknown and untraceable.

Thus, addressing fraud should focus on being proactive in prevention and detection. Raw material monitoring should be performed using appropriate analytical methods for the verification of authenticity. Our laboratory analyzed a total of 425 beef products to discover that a significant proportion of them had been adulterated with water buffalo meat, *Bubalus bubalis*.

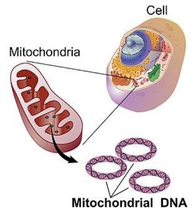
MATERIALS AND METHODS

A total of 425 beef products submitted between March and July 2019 by an anonymous meat manufacturer were included in the study.

For genetic analysis, DNA was extracted from each meat sample using a commercial **GMO Extraction Kit** (Thermo Fisher Scientific). To ensure optimal representativeness of the sample, 200 g of raw meat were homogenized from which 10 g were incubated in lysis buffer, proteinase K and RNase reagent according to the manufacturer's instructions.

The quality and quantity of the purified DNA sample was studied by spectrophotometry. The analytical monitoring was performed by real-time PCR using the 7500 FAST Real-Time PCR System (Applied Biosystems) and screening kit **Imegen™ Water Buffalo ID Kit** (Imegen). For this, a total amount of 50 ng of total DNA were used to set up the PCR reaction. Only when the screening was positive, quantification of buffalo DNA was performed using **RapidFinder™ Quant MultiMeat Set** (Thermo Fisher Scientific). All samples were extracted and analyzed in duplicate. A total amount of 50 ng of total DNA were used to set up the PCR reaction.

The buffalo amplification system targets a highly specific mitochondrial DNA region, which confers great specificity and sensitivity (limit of detection established at 0.01%).



Standard curves were constructed to calculate the copy number of buffalo DNA versus the totality of animal DNA copies. Animal DNA was quantified from conserved *16S* gene.

RESULTS

Our results indicated that all DNA samples were suitable for the analytical study. Total DNA concentration ranged between 100 to 250 ng/µL and the 260/280 and 260/230 wavelength ratios were >1.8 in all samples suggesting high purity and absence of PCR inhibitors.

Diagnostic detection of buffalo DNA using Real-Time PCR was interpreted based on a cycle threshold (Ct) value [1]. The cut-off value above which a Ct value is deemed false, was estimated with the following formula:

$$Ct_{cut\text{-}off} = 3.32 + Ct_{Positive\ Control}$$

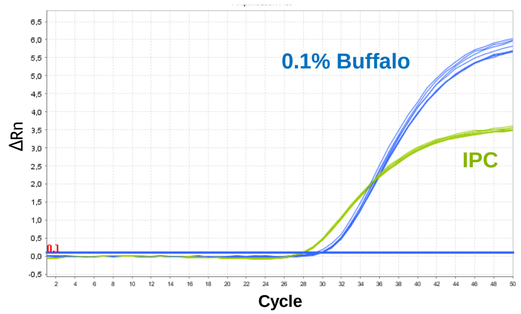


Fig 1. Real-Time PCR amplification plot. A) Detection results obtained from the positive control included in Imegen-Water Buffalo ID Kit. Buffalo DNA was labelled with FAM, and the internal positive control (IPC) with VIC. The IPC consists of a synthetic DNA region directed to function as a PCR control to confirm the correct set up and functioning of the PCR.

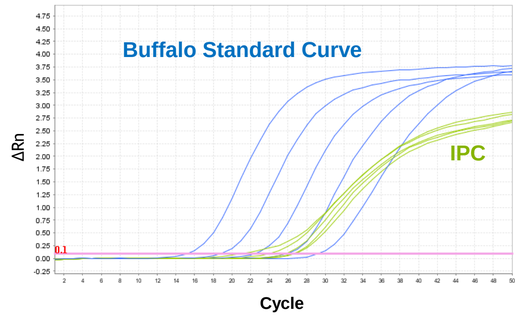


Fig 2. Standard curve constructed using RapidFinder™ Quant MultiMeat Set used to calculate the total number of DNA copies.

The first batch of samples studied included 21 meat products containing > 80% buffalo meat.

Since the horse meat scandal, the EU Commission released a recommendation indicating follow-up controls shall be performed [2,3] and the removal of debris and product changes must be ensured to comply the acceptability limit of < 1%.

Analytical surveillance elucidated that 8.2% of beef products were adulterated with > 1% buffalo meat.

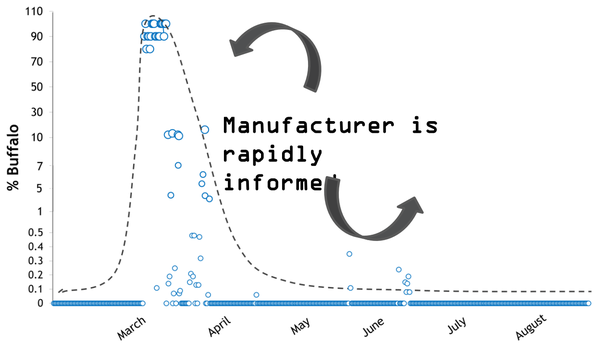


Fig 2. Graphic representation of the 425 beef samples analyzed from March to August 2019

CONCLUSIONS

Analytical monitoring successfully detected that beef products were being adulterated with water buffalo meat. Following notification to the manufacturer, insignificant amounts of buffalo were identified. The results suggest preventive measures were taken to ensure the product authenticity.

Overall, the present study highlights the effectiveness of implementing analytical surveillance to ensure the authenticity of food products by minimizing vulnerability to fraud using the most appropriate method.

REFERENCES

1. **Caraguel CGB et al.** Selection of a Cut-off Value for Real-Time Polymerase Chain Reaction Results to Fit a Diagnostic Purpose: Analytical and Epidemiologic Approaches. J Vet Diagnostic Investig. SAGE PublicationsSage CA: Los Angeles, CA; 2011;23: 2–15. doi:10.1177/104063871102300102
2. **Stadler RH et al.** Analytical Approaches to Verify Food Integrity: Needs and Challenges. J AOAC Int. 2016;99: 1135–1144. doi:10.5740/jaoacint.16-0231
3. **2013/99/EU: Commission Recommendation** of 19 February 2013 on a coordinated control plan with a view to establish the prevalence of fraudulent practices in the marketing of certain foods. http://data.europa.eu/eli/reco/2013/99/oj

TRADEMARKS/LICENSING

LT2491A September 2019

imegenagro ThermoFisher SCIENTIFIC

thermoscientific

Food Safety Monitoring: The Use of Specific Swine Detection Methods to Ensure Halal Authenticity

Jennifer Valero-Garcia[1], Greta Carmona-Antoñanzas[1], Amanda Manolis [2], Marta Izquierdo-García[1] , Yolanda Pérez-Estarelles[1], Merche Bermejo-Villodre[1], Carlos Ruíz-Lafora[1]

[1]Instituto de Medicina Genómica, Calle Agustín Escardino 9, Parc Científic de la Universitat de València [2]Thermo Fisher Scientific, Microbiology, Basingstoke, RG24 8PW, UK

ABSTRACT

In the past few decades, Halal meat has had growing sales with Muslim communities totalling nearly 25% of the world population. The qualification of Halal, permitted as per Islamic Shari'ah, addresses attributes that refer to the method of production and establishes that products must be free of any prohibited ingredients, such as pork, animals slaughtered improperly and other intoxicants. Despite preventive measures, food industries might fail to produce food which is not correctly described and may be contaminated with pork derivatives. Analytical tests in meat have increased in recent years due to the discovery of species adulteration in processed products.

To ensure Halal authenticity, food safety enforcement authorities perform controls at each stage of the agri-food chain, and Halal entities are responsible of certifying goods apt for consumption by Muslims through coherent measures and adequate analytical monitoring. Our laboratory analysed a total of 507 samples supposed to be Halal using a highly sensitive analytical method (sensitivity > 0.0005%) to discover that a significant proportion of the samples analysed presented traces of pork DNA. Such small amounts of pork DNA might end up adulterating the final products due to accidental contamination during processing, thus rendering it Haram, or non-permitted.

The present study highlights the importance of implementing specific and sensitive analytical surveillance methods to ensure the authenticity of Halal products.

INTRODUCTION

In the past few decades, Halal meat has had growing sales with Muslim communities totaling nearly 25% of the world population. The qualification of Halal, permitted as per Islamic Shari'ah, addresses attributes that refer to the method of production and establishes that products must be free of any prohibited ingredients, such as pork, animals slaughtered improperly and other intoxicants.

To ensure Halal authenticity, food safety enforcement authorities perform controls at each stage of the agri-food chain, and Halal entities are responsible of certifying goods apt for consumption by Muslims through coherent measures and adequate analytical monitoring.

Our laboratory analyzed a total of 520 samples supposed to be Halal using a highly sensitive analytical method (sensitivity > 0.0005%).

MATERIALS AND METHODS

A total of 520 Halal products submitted between January 2018 and June 2019 by multiple meat manufacturer were included in the study.

For genetic analysis, DNA was extracted from each meat sample using a commercial **GMO Extraction kit** (Thermo Fisher Scientific). To ensure optimal representativeness of the sample, 200 g of raw meat were homogenized from which 10 g were incubated in lysis buffer, proteinase K and RNase reagent according to the manufacturer's instructions.

The quality and quantity of the purified DNA sample was studied by spectrophotometry. The analytical monitoring was performed by Real-Time PCR using the 7500 Fast Real-Time PCR Food Safety System (Thermo Fisher Scientific) and screening kit RapidFinder™ Pork ID kit and RapidFinder™ Halal ID kit (Thermo Fisher Scientific). The swine amplification systems target highly specific mitochondrial DNA regions, which confer great specificity and sensitivity (limit of detection established at 0.01% and 0.0005%, equivalent to 5 ppm), respectively.

For this, a total amount of 50 ng of total DNA were used to set up the PCR reaction. All samples were extracted and analyzed in duplicate.

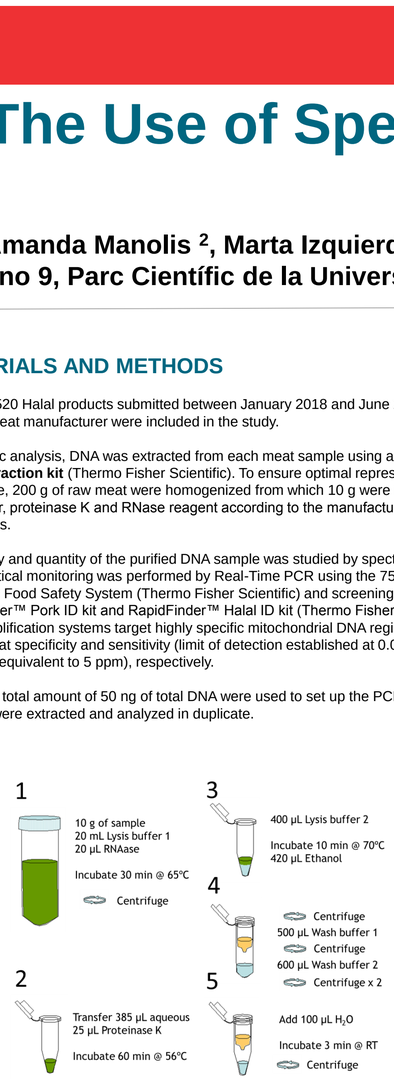


Figure 1. DNA extraction protocol to ensure a high quality and purity DNA sample.

RESULTS

Our results indicated that all DNA samples were optimal for the genetic analysis. The **GMO Extraction kit** is optimal for low-target detection in assays that require great sensitivity as it processes over 20-times more sample than most commercial kits. In addition, it yields high DNA concentration (> 100 ng/µL) and purity suitable for any PCR-based downstream assays.

RapidFinder Halal ID kit is a highly sensitive system capable to detect 5 ppm of pork DNA. This sensitivity allows meat manufacturer to reliably label and trace the authenticity of the sample. Diagnostic detection of pork DNA using Real-Time PCR is interpreted based on a cycle threshold (C_t) value[1]. The cut-off value is stablished on 0.0005% above which a C_t value is deemed false.

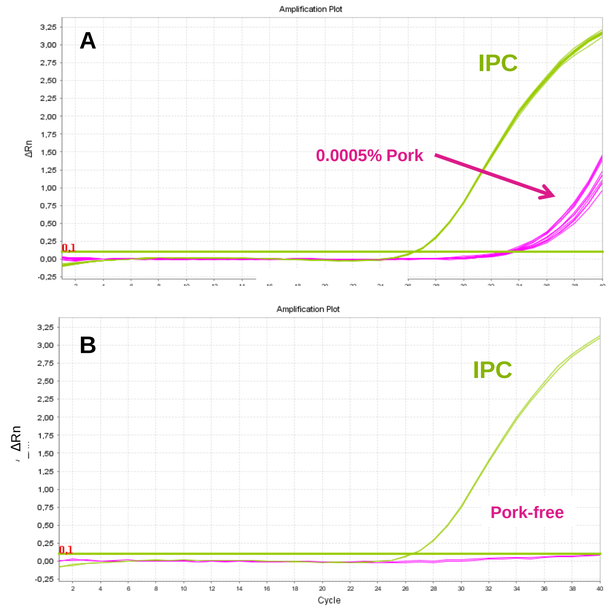


Figure 2. Real-Time PCR amplification plot. A) Detection results obtained from a DNA sample containing 0.0005% (5 ppm) swine DNA. Swine DNA is labelled with FAM, and the internal positive control (IPC) with VIC. The IPC consists of a synthetic DNA region directed to function as a PCR control to confirm the correct set up and functioning of the PCR; B) Swine-free sample suitable for Halal labelling.

Overall, 15% of the samples contained pork DNA. Among them 21 were only detected by the Halal-specific assay with a sensibility of 0.0005% (5 ppm).

Analytical surveillance confirmed that 85% of the Halal products were free of pork. Overall, 78 samples contained traces of pork.

Follow-up monitoring informs the Halal product manufacturers of a breakage in the cleaning and decontamination process in the production chain enabling them to seek solution to mitigate cross-contamination with pork-containing products.

The EU Commission released a recommendation indicating follow-up controls shall be performed [2,3] and the removal of debris and product changes must be ensured to comply the acceptability limit of < 1% accidental contamination, guidelines of Halal requirements vary between country authorities.

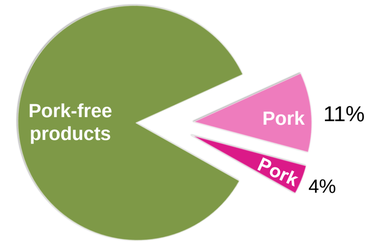


Figure 3. Graphic representations of the 520 Halal products analyzed. Light pink, samples detected with RapidFinder Pork ID kit; Dark pink, samples detected with highly sensitive (5 ppm) RapidFinder Halal ID kit.

CONCLUSIONS

The present study indicated that a highly sensitive analytical tool is capable to detect traces of swine DNA to ensure the authenticity of Halal products. Both genetic assays successfully detected swine DNA at concentrations below the EU Commission recommendation to prevent food fraud.

In conclusion, the present study highlights the effectiveness of implementing analytical surveillance to ensure the authenticity of food products by minimizing accidental contamination.

REFERENCES

1. Caraguel CGB et al. Selection of a Cut-off Value for Real-Time Polymerase Chain Reaction Results to Fit a Diagnostic Purpose: Analytical and Epidemiologic Approaches. J Vet Diagnostic Investig. SAGE Publications Sage CA: Los Angeles, CA; 2011;23: 2–15. doi:10.1177/104063871102300102
2. Stadler RH et al. Analytical Approaches to Verify Food Integrity: Needs and Challenges. J AOAC Int. 2016;99: 1135–1144. doi:10.5740/jaoacint.16-0231
3. 2013/99/EU: Commission Recommendation of 19 February 2013 on a coordinated control plan with a view to establish the prevalence of fraudulent practices in the marketing of certain foods. http://data.europa.eu/eli/reco/2013/99/oj

TRADEMARKS/LICENSING

LT2490A September 2019

imegenagro

ThermoFisher SCIENTIFIC