**Meat forensics analysis of fast food chains located at the University of British Columbia**

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**Abstract**

Increased demand for meat products has caused the food industry worldwide to amplify production, leading to malpractice and meat fraud around world supply chains. This study aimed to assess beef quality among burgers on the University of British Columbia (UBC) campus for undeclared meat species. It was expected that the burgers sampled would contain the presence of undeclared species due to cross-contamination in the workplace. DNA extraction and multiplex PCR were conducted on 5 burgers across the University of British Columbia (UBC) campus including a plant-based burger, and raw steak as a control. The positive control indicated the presence of cattle (274bp) and horse meat (439bp). Results among other samples were inconsistent due to smearing spanning cattle (274bp), poultry (398bp), and horsemeat (439bp). Contamination of the control was suspected to be the result of non-sterile techniques used during production. Photospectrometry confirmed protein contamination (~1.70 avg) among most samples, suggesting heat-induced DNA degradation during the cooking process and compromising the effectiveness of PCR amplification. Future investigations would suggest the analysis of raw meat to mitigate complications of heat-induced DNA damage on PCR amplification.

**Introduction**

Throughout the world, red meat serves as a key ingredient in many different cuisines and diets. As a result, red meat production and consumption have increased dramatically over the past decade. According to World Consumption of Meat, red meat production has "increased 5 times in the second half of the 20th century", and is estimated to increase by another 160%. With the growing demand for meat products, the industry has had to scale up and automate its production systems. This rapid expansion has led to products being much more prone to cross-contamination and/or mislabelling. These are unintentional mistakes that impact the quality of foods we consume. However, there are also many cases of meat fraud throughout history, such as the Horsemeat Scandal of 2013, which has "highlighted vulnerabilities within the European beef supply chain" (Robson et al., 2020). Such cases of mislabelling and mishandling meat

products raise major religious, ethical, and economic concerns. In a study, 7.27% of meat products tested were found to be contaminated with prohibited meats according to Islamic law, such as pig, horse, and donkey (Doosti et al., 2014). In addition, such meat adulterations have economic implications as failure to accurately label meat products can lead to financial losses impacting both consumers and producers (Doosti et al., 2014).

The purpose of this paper is to assess the beef meat quality of common fast food chains located at the University of British Columbia (UBC) and to determine if there is any presence of other meat types in beef products. This analysis is done using the PCR method, which is a widely used DNA-based identification technique involving polymerase chain reactions (Rahmati et al., 2016). There is an abundance of research on this topic, many of which were reviewed. A study conducted by Dalsecco et al. in 2018, using the PCR method, revealed that out of 14 commercial meat products, 6 samples had non-declared bovine and/or chicken presence. These results are parallel to findings in other literature, including papers by Dawn & Hellberg (2016), Di Pinto et al. (2015), Doosti et al. (2014), and López-Maestresalas et al (2019). Due to the high number of contamination cases across these studies, we hypothesize that some of the burgers sampled in our experiment will have the presence of undeclared species. Similarly, the plant-based burger samples are predicted to report the presence of cattle, chicken, or pig, and samples of beef burgers are predicted to report the presence of chicken or pork after conducting multiplex PCR.

## Methods

Five cooked burgers were obtained from four different fast food chains located within the UBC Vancouver campus; one beef burger was bought from each restaurant as well as a single

plant-based burger. From each burger, three samples were collected randomly across the patty. Additionally, a cut of raw steak was collected for use as a positive control.

DNA extraction was performed on the 16 samples; 300 µL of Cell Lysis Solution with Proteinase K to each sample and incubated at 65ºC for 15 minutes. 150 µL of protein precipitate reagent was added, samples were centrifuged, and the supernatants were transferred to new tubes. 500 µL of isopropanol was used to precipitate the DNA. Samples were centrifuged once again, and the DNA pellet was washed twice with ethanol and finally resuspended in 30 µL of TE buffer.

Multiplex PCR was then conducted using the protocol described by Matsunaga et al. (1999), in which the cytochrome b region of mitochondrial DNA was the amplified region of interest. 25 µL reactions were set up, containing PCR buffer, 1.5 mM MgCl2, 200 µM dNTP mix, primers (0.4 µM SIM, 0.24 µM cattle, 0.24 µM pig, 1.2 µM chicken, 1.2 µM sheep, 0.08 µM goat, and 0.8 µM horse), 10% glycerol, 2.5 U Taq polymerase, and 1 µL of extracted DNA or dH2O. The thermocycling program used was described by Matsunaga et al. (1999). Amplified products and a mix of DNA ladders (BioShop, Ultra Low) were run on a 3% agarose gel at 120 V for 60 min. The expected DNA fragment sizes for goat, chicken, cattle, sheep, pig, and horse meats are 157, 227, 274, 331, 398, and 439 bp, respectively (Matsunaga et al., 1999).

DNA spectrophotometry was performed on the extracted DNA of one randomly selected sample from each restaurant as well as the positive control. Extracted DNA samples were diluted by a factor of 200 in $dH_2O$ prior to measurement. The DNA concentration and absorbances at wavelengths of 260 nm and 280 nm were recorded.

## Results

The observational study determined that there was indeed meat contamination within the positive control but other results were mainly inconclusive. The DNA-PCR analysis shows bands at 157bp representing goat, 227bp representing chicken, 274bp representing cattle, 331bp representing sheep, 398bp representing pig, and 439bp representing horse meat.

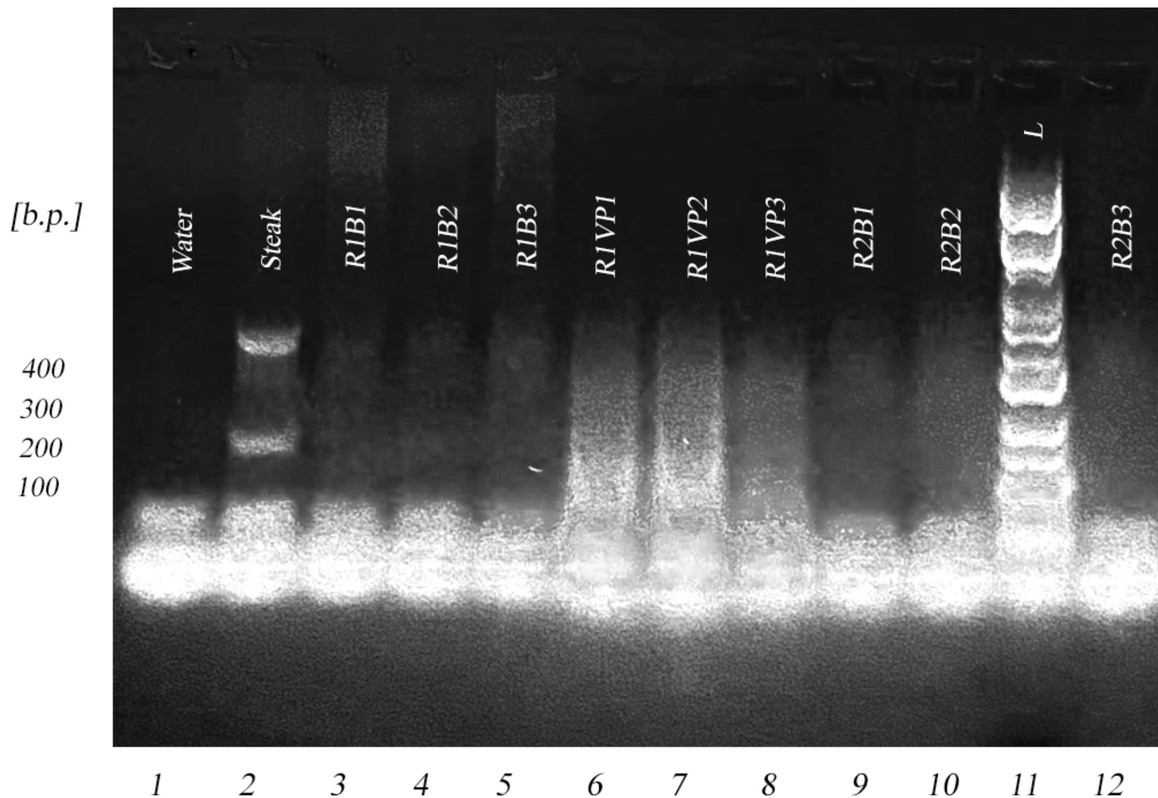


**Figure 1.** *The figure shows the negative control as water, positive control as raw beef steak, R1 as restaurant 1, VP as a vegan patty, R2 as restaurant two, B1 as beef patty one, B2 as beef patty two, and B3 as beef patty three. 1, 2, and 3 were labeled for beyond meat patties one, two and three. The X-axis represents the variables and the y-axis represents the bands with bp.*

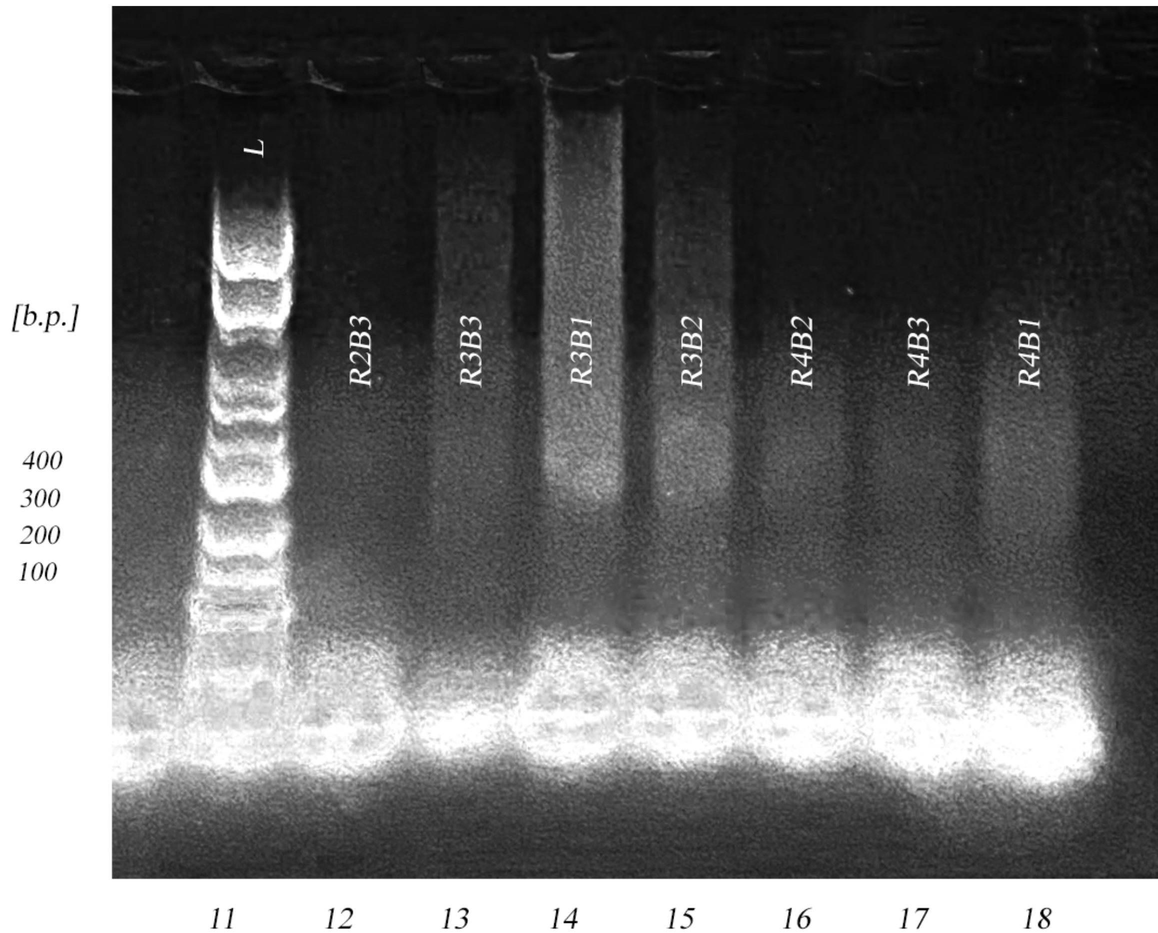


**Figure 2**. *R2 indicates restaurant two beef burgers, R3 indicates restaurant three beef burgers, R4 indicates restaurant four beef burgers, and 1,2,3 represent the 1,2,3 sampled beef burger patties, respectively. The x-axis represents the variables and the y-axis represents bands in bp.*

The figure demonstrates no identification of DNA bands in water. The species-specific DNA fragment showed three bands for the positive control: a visible band at 274bp indicating cattle, a visible band at 439bp indicating horse meat, and a faint band at 398bp indicating poultry. Restaurant 1's meat burgers had faint bands of cattle at a species-specific DNA band at 274bp, poultry at 398bp, and horsemeat at 439bp. The vegan patties show no visible meat bands seen from the long length of DNA bands, indicating various proteins. Restaurant 2's beef burger

patties show no evident bands. There were no specific bands at Restaurant 3; only a large band of proteins was seen. No visible bands are also seen at Restaurant 4 across all the patties.

DNA spectrophotometry was performed to assess the quality of extracted DNA using 260nm/280nm ratios. Ratios larger than 1.8 indicated RNA contamination and ratios smaller than 1.8 indicated DNA contamination. As per Figure 3, the positive control indicated RNA contamination with a 1.92 ratio. Restaurant 1 meat had a 1.71 ratio. Restaurant 1's vegan patty ratio is 1.89. Restaurant 2 meat had a ratio of 1.49. Restaurant 3 beef had a ratio of 1.59. Restaurant 4 beef had a ratio of 1.58.

| Variable | Positive Control | Restaurant 1 Meat | Restaurant 1 Vegetarian Patty | Restaurant 2 Meat | Restaurant 3 Beef | Restaurant 4 Beef |
|---|---|---|---|---|---|---|
| Concentration (ug/ml) | 433 | 927 | 6431 | 1841<br>1.49 | 2010 | 920 |
| Absorbance at 260nm/ Absorbance at 280nm | 1.92 | 1.71 | 1.89 | 1.49 | 1.59 | 1.58 |

**Figure 3:** *The various DNA spectrometry for the meat samples. The samples showed DNA and RNA contamination across the meat samples.*

**Discussion**

Our results were inconclusive as there were no distinct bands present on the gel electrophoresis, except for the positive control, to indicate the presence of meat species within the burger samples. Therefore, we were unable to address the aim of the study to determine whether unlabeled meat species, including goat, chicken, pig, or horse meat were present in burgers sold by restaurants on the UBC Vancouver campus.

Given that bands were present for the raw meat (positive control) sample but not for any of the cooked burger samples, it is likely that DNA degradation, resulting from the heat of cooking, interfered with PCR. In a study by Jun et al. (2023), the integrity of DNA in raw versus cooked beef was investigated. Their findings suggest cooking causes a great deal of hydrolytic and oxidative damage to all four bases of DNA (Jun et al., 2023). More specifically, heat accelerates the deamination of 2′-deoxycytidine (dC) to 2′-deoxyuridine (dU) in DNA and the oxidation of guanine, forming 8-oxo-2′-deoxyguanosine (8-oxo-dG) as well as other modified deoxynucleosides (Jun et al, 2023). This said alteration and damage to DNA bases of cooked samples in this study could have interfered with primer binding to the degraded DNA during PCR, therefore explaining the absence of distinct bands on the gel.

Spectrophotometry results indicate that there is a sufficient concentration of template DNA present for all samples for PCR. This supports the claim that PCR amplification failure was due to DNA damage rather than the absence of a DNA template altogether. Furthermore, samples containing pure DNA have a ratio of absorbance at 260 nm ($A_{260}$) to absorbance at 280 nm ($A_{280}$) of 1.8. A ratio higher than 1.8 is suggestive of RNA contamination and a ratio below 1.8 suggests that the sample is protein-contaminated (Lucena-Aguilar et al., 2016). This said burger samples from restaurants 1, 2, 3, and 4 are protein-contaminated. On the other hand, the positive control and vegetarian patty from Restaurant 1 are RNA-contaminated. This is most likely due to non-sterile techniques during the conduction of this experiment. The protein and RNA contaminations in all samples explain why there is a consistent smear on the gel across all samples (“How to interpret agarose gel data”, n.d.; Gallego, n.d.).

Our positive control, raw steak sample, surprisingly produced two bands indicative of the presence of cattle and horse meat. The aim with the positive control was for the sample to only

showcase one band corresponding to cattle. However, we believe the presence of the band corresponding to the horse species is likely not because of the presence of horse meat in the sample but rather due to DNA ladder bands not separating properly. This could be due to inadequate gel running conditions: insufficient agarose concentration for the DNA sizes, insufficient power supply, and shorter than optimal duration of running the gel (Gallego, n.d.). It is much more likely that the second meat species present within the positive control is a different meat species (e.g. pig) rather than horse meat. Regardless, the presence of a second species than cattle in the store-bought steak is concerning.

In the study conducted by Matsunaga et al. (1999), from which we adopted our multiplex PCR methodology, they successfully amplified cattle, pig, chicken, sheep, and goat DNA fragments from meat cooked at 100 or 120 °C for 30 min. This is in contrast to the results from this study. These inconsistencies could be due to differences in the heating temperature and duration of cooking of the burgers by the restaurants compared to the heating protocol used by Matsunaga et al. (1999). It is possible that the restaurant-cooked burger DNA was subject to more DNA degradation. It is worth mentioning that in the study conducted by Jun et al. (2023), beef samples were cooked at 100 °C for 30 minutes and significant DNA damage was noted. This supports the claim that the duration of cooking could change the level of DNA damage and hence impact PCR products.

Overall, the findings from this experiment are inconclusive as to whether the burger samples from restaurants on the UBC campus are contaminated with unlabeled meat species, and whether food fraud is exhibited or not. For future studies, to improve our experimental procedure to yield distinct bands, we will use raw rather than cooked burger samples to eliminate the negative impact of heat-induced DNA damage on PCR amplification of DNA.

**Conclusion**

Burger samples were collected across multiple restaurants across UBC campus, including beef and plant-based burgers, as well as raw steak. Among the burger samples, the presence of contamination remains unclear, due to consistent smearing among gel electrophoresis bands. Such smearing is suspected of primers in the experimental design (Matsunaga et al. 1998) being compromised by DNA degradation inhibiting the efficiency of PCR amplification, due to temperature and cooking times resulting in protein contamination. (Jun et al. 2023) The positive control however produced a distinguishable band pattern, indicating the presence of cattle and horse meat. The contaminated control was suggested to be the result of non-sterile techniques throughout production. To minimize DNA damage in subsequent procedures, the analysis of raw products for all samples is suggested to mitigate the impacts of DNA degradation due to temperature and cooking duration.

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