Meat Mislabelling in Sausages: A Detailed Exploration Using DNA Analysis Techniques for Product Verification

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Abstract

Accurate food labelling is crucial for both food safety and consumer awareness, especially for sausages which are a major protein source for people worldwide. Our study examined a total of 6 different sausage samples: 2 beef, 2 pork, and 2 chicken. Meat primers used for the multiplex PCR procedure were cattle (beef), pig (pork), chicken, sheep, goat, and horse. We hypothesized that 1 out of 6 of the sausage samples would contain undeclared meat species based on the rates of sausage meat contamination in previous Canadian studies (Naaum et al., 2018; Shehata et al., 2019). Our results concluded that at least 16.67% of the 18 sausage samples contained undeclared meat species. Contamination was only found in pork sausage samples which additionally had horse, chicken and cattle DNA detected. While we expected to find a reduced rate of mislabelling compared to a decade ago, the detection of horse meat suggests that new sources of contamination may be emerging. Overall, these findings indicate that the prevalence of undeclared meat species in sausage products persists in the food industry given the health, cultural, and transparency issues involved.

Introduction

The primary objective of our study is to contribute to the ongoing monitoring of mislabelling rates in sausage products and to assess whether the improvements observed between the Naaum et al. (2018) and Shehata et al. (2019) studies have been sustained in the years since. Through our research, we hope to provide valuable insights into the current state of meat product labelling and contribute to the broader discourse on food safety, consumer trust, and industry accountability.

Consumer trust in the accuracy of food labelling is essential for making informed choices based on health, cultural, religious, and personal beliefs. In recent years, concerns have arisen regarding the mislabelling of meat products, particularly sausages, where the ground and processed nature of the meat makes it difficult to identify its origin. Mislabelling can result from

economic motivations, cross-contamination during processing, or simple error, but in all cases, it poses risks to consumer health, introduces potential food pathogens, and undermines the integrity of the food industry. While previous studies have investigated the prevalence of mislabelling in sausage products, there remains a need for continued monitoring to assess the effectiveness of industry regulations and the persistence of this issue.

In this paper, we present the results of our investigation into the presence of undeclared species in sausage products from multiple brands sold at a local grocery store. From two packs of chicken sausage, pork sausage, and beef sausage, we collected 3 replicates giving us a total of 18 sausages which were examined using DNA analysis techniques. Our study builds on the findings of a key research paper by Naaum et al. (2018) and its follow-up by Shehata et al. (2019). These studies employed DNA barcoding and PCR methods to detect undeclared species in sausage samples from Canadian retail markets. These studies found mislabelling rates of 20% and 14%, respectively, indicating a reduction in mislabelling over time but highlighting the ongoing nature of the problem. We define contamination and mislabelling as the presence of any undeclared species, which is in line with the Canadian labelling requirements and guidelines for meat products (Canada Food Inspection Agency, 2022).

Based on the rates of meat mislabelling found by the aforementioned studies, we expect that at least one pack out of the six would have contaminants from other meats and thus be mislabelled. Depending on the type and severity of this contamination, we may also be able to deduce whether or not such contamination was accidental like a supply chain issue or intentional in that the company is deliberately mixing undeclared species in the sausages to maximize profit.

Methods

The meat content of sausages was analyzed through a series of experiments. To start, 6 sausage packs were collected from a grocery store, each representing a different type of meat and brand of sausage. There were 2 chicken, 2 beef, and 2 pork sausage packs and 3 replicates for each type of sausage were made to have a total of 18 sausage samples. DNA from each sample was extracted by adding a small amount of each sausage to a labelled 1.5ml Eppendorf tube, mashing it with a sterile toothpick, and adding 300µl of Cell Lysis solution with Proteinase K. The tubes were then vortexed at maximum speed for 10 seconds before incubating them at 65°C for 15 minutes.

While being incubated, tubes were taken out every 5 minutes and vortexed for 10 seconds. After incubation, the samples were placed into an ice bath for 5 minutes, and 150 µl of Protein Precipitate Reagent was added to each tube. After vortexing for 10 seconds, the samples were placed into a centrifuge at a maximum speed of 13.0 revolutions per minute (RPM) for 10 minutes. The supernatant from each tube was transferred to a sterile Eppendorf tube while the pellet and fat were discarded from each sample. 500µl of ice-cold isopropanol was then added and each tube was inverted 30-40 times. The samples were centrifuged at maximum speed (13.0 RPM) for another 10 minutes. The liquid from each sample was removed without disturbing the pellet at the bottom of each tube. Finally, 500µl of ethanol was added to each sample and then taken out with a pipette to remove any excess salts. The sausage samples were then left overnight with their caps open to dry.

To assess the meat content of the sausages, a polymerase chain reaction (PCR) was performed. The Master Mix (MM) was scaled up 20 times to give a total volume of 480µl to reduce additional pipetting steps and possible errors. This accounted for the 18 sausage samples, 1 negative control, and an additional sample in case extra MM was needed. The MM that was used was taken from the Matsunaga et. al (1999) multiplex PCR approach, where primers for a certain type of meat are added. PCR samples consisted of 6 specific meat primers: 157 base pairs (bp)-goat, 227 bp-chicken, 274 bp-beef, 331 bp-sheep, 398 bp-pig and 439 bp-horse. The ladder (L) ranged from 100 to 300 base pairs (100, 150, 200, and 300). 72ul of deionized water was first added to the MM, followed by 10X PCR Buffer (50µl), 10nM dNTPs (10µl), 25mM $MgCl_2$ (30µl), 50% Glycerol (100µl), and Taq polymerase (10µl). The meat forward, chicken, goat, beef, pig, sheep, and horse primers were also added at 20µl, 60µl, 4µl, 12µl, 12µl, 60µl, and 40µl respectively. Then, 24µl of the MM was added to 19 PCR tubes (including 1 negative control). Finally, 1ul of the isolated DNA (that had been resuspended with 30µl of TE buffer a few hours before our PCR experiment) was added to all 18 of the PCR tubes and 1µl of deionized water was added to the single negative control tube.

PCR was conducted using a cycle of 95°C for 2min, followed by 35 repeats of 95°C for 30 sec, 60°C for 30 sec, 72°C for 30 sec, and lastly 72°C for 5 minutes (Figure 1). Samples were then kept at 4°C overnight and stored in the freezer until further use.

95°C 2 min.
95°C 30 sec.
60°C 30 sec.
72°C 30 sec. } 35x
72°C 5 min.
4°C overnight
Store in freezer

Figure 1. PCR cycle detailing the temperatures of the denaturation, annealing, and extension steps.

Lastly, gel electrophoresis was used to analyze the PCR products. To each PCR sample, 5µl of 6X loading dye was added and 10µl of each sample was added to the wells of a 3% agarose gel, with a negative control on the very left and a ladder corresponding to 100, 150, 200, and 300 base pairs in the middle. The samples were run on the gel at 120V for an hour, and the results were ready to be analyzed.

Results

All sausage samples showed prominent bands at their respective expected base pair counts, but the P 2 samples showed additional bands. P 2.1 had a lower band matching the chicken band (227 bp). P 2.1, P 2.2, and P 2.3 also had an additional band matching the 439 bp, horse primer.

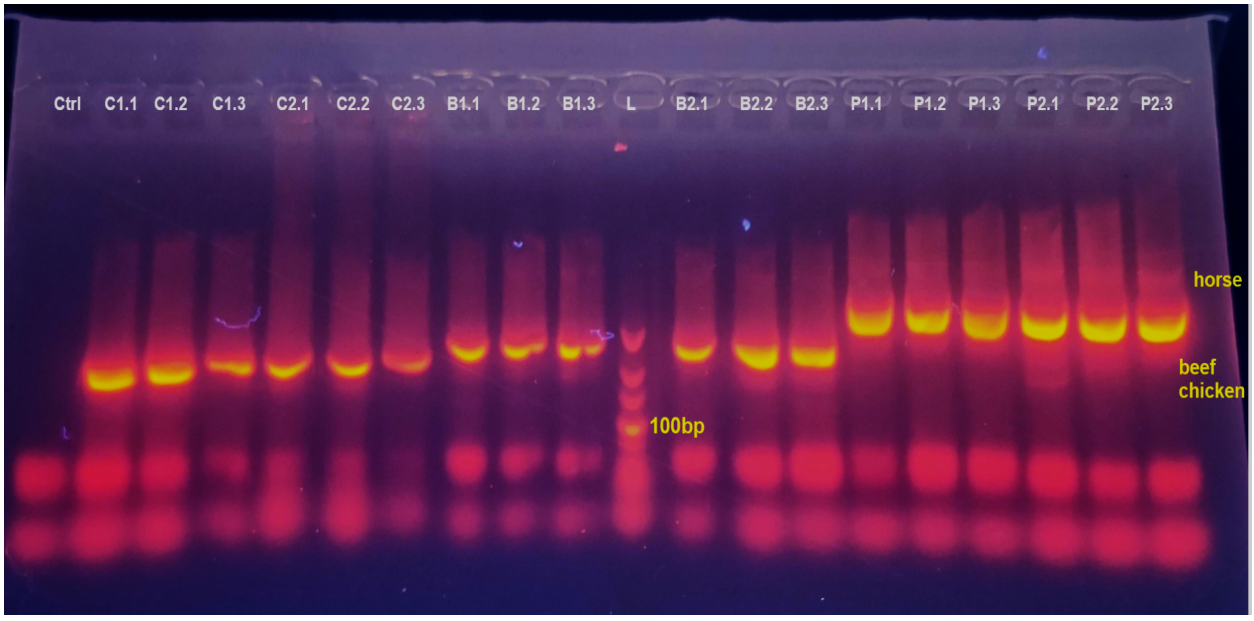

Figure 2. Result of PCR samples loaded on 3% agarose gel. Abbreviations stated for the loading order Ctrl, C, B, L, and P stand for control, chicken, beef, ladder, and pork respectively.

The total mislabelled rate calculated from the 18 sausage samples was 16.667% in total (Table 1). There were a few samples that had ambiguous bands. B 2.2 and 2.3 had ambiguous bands matching the chicken primer and P 2.1, P 2.2, and P 2.3 had ambiguous bands matching the beef primer. For this analysis, ambiguous bands were not considered to be mislabelled.

Sausage Samples	**Mislabelled**	**No Mislabelling Detected**	**Mislabelled Rate**
C 1	0	3	0%
C 2	0	3	0%
B 1	0	3	0%
B 2*	0	3	0%
P 1	0	3	0%
P 2**	3	0	100%
Total Mislabelled Rate			16.667%

Table 1. Data observed from Figure 2. All 6 sausage samples had 3 replicates, a total of 18 samples. Abbreviations C, B, and P stand for chicken, beef, and pork respectively.
* Ambiguous presence of a band matching the chicken primer for B 2.2 and B 2.3.
** Ambiguous presence of another band matching the beef primer for P 2.1, P 2.2, and P 2.3.

Discussion

The existence of at least one instance of mislabelling in our sample of six sausage types is in line with previous studies on Canadian sausage products (Naaum et al., 2018; Shehata et al., 2019). This supports our hypothesis.

Interestingly, our study found that pork sausages were the most likely to have undeclared species (Table 1). This differs from a previous Canadian study that found beef sausages to be particularly susceptible to meat adulteration due to their relatively high cost and the availability of cheaper meat substitutes such as pork (Naaum et al., 2018). However, one out of 38 pork sausage samples in Naaum et al.'s study was contaminated with horse meat which raises the question of whether horse meat contamination is an emerging issue in the meat industry. Tareq Al-Qassab et al. (2019) found that there was a high rate of undeclared chicken meat compared to red meat found in their sausage samples likely due to economic reasons because chicken is cheaper than red meats such as beef and pork.

While one of the pork sausage replicates showed a band indicating the presence of chicken DNA, it is not certain that this is a case of mislabelling and not contamination. During the experiment, contamination may have occurred due to no chicken bands being present in other samples of the same sausage type. Further experimentation would be required to conclude whether the P2 sausage samples have chicken contamination. That being said, we were able to distinguish bands that had a greater base pair length than 398 in our P2 samples. The only tested species with a DNA base pair length greater than pork is horse DNA which we deduced from the process of

elimination. We had no samples that contained horse meat in their ingredient lists, which implies this is a case of contamination or mislabelling.

It is worth noting that horse meat can be a cheaper alternative to pork, and it is a traditional ingredient in sausages for some cultures. However, the use of undeclared horse meat in sausage products is illegal in Canada and other countries. Additionally, it can pose potential health risks to consumers who may have allergies or religious or cultural restrictions against consuming horse meat.

Also, one of the P2 replicates had a defined band of chicken DNA. Since it only showed in one out of three samples though it could be a result of our own contamination. Inadequate sterilization of tongs and scissors between sample collection is suspected to be the cause of the chicken contamination. There were also two faint bands detected indicating the presence of beef in the P2 sausage samples but the bands were faint, so it is ambiguous whether these are contaminants.

Further experimentation to address the uncertainty related to poor band quality would be to test for only one type of DNA in a sausage brand and observe whether there is a band or not. Alternatively, a separate master mix could be used for each type of sausage, excluding the type of primer that matches what should be in the sausage. For example, the master mix used for pork sausages would have the pork primer omitted but retain the other types of meat primers. This would allow bands indicating contamination to be more visible as the main band would not mask

the contaminant bands. The results for this study may have potential contaminant bands but their presence was ambiguous as the expected band for each sample was significantly bolder.

Overall, our study adds to the body of evidence indicating that meat adulteration is a significant issue in the food industry. The prevalence of undeclared species in sausages highlights the need for more rigorous quality control measures and increased regulation to ensure that food products are safe and meet consumers' expectations.

Conclusion

Our study aimed to contribute to the ongoing monitoring of mislabelling rates in sausage products and to assess whether the improvements observed between the 2018 and 2019 studies have been sustained in the years since. Of the 6 different sausage types tested, one type of pork sausage was clearly contaminated with horse meat in all three replicates and they also had ambiguous bands that we suspect to be contamination of beef and chicken. Two beef sausage replicates also showed ambiguous lines suspected to be chicken contamination. In light of these results, it is clear that food mislabelling and contamination continue to be an issue for sausage products sold in Canada.

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Appendix

Master mix preparation for 20 samples (including 1 negative control and 1 backup)

Solution	Volume (ul) (scaled up to 20x)
10X PCR Buffer	50
10nM dNTPs	10
25mM MgCl2	30
5’ Primer 10uM (Meat forward (SIM)	20
3’ Primer 10uM (Goat “G”)	4
3’ Primer 10uM (Chicken “C)	60
3’ Primer 10uM (Cattle “B)	12
3’ Primer 10uM (Sheep “S”)	60
3’ Primer 10uM (Pig “P”)	12
3’ Primer 10uM (Horse “H)	40
Taq Polymerase	10
50 % Glycerol	100
Total	480 (including initial dH2O)