DNA Analysis on Metro-Vancouver Sausages

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Abstract:

This study investigated the labelling of sausage meats in the Metro Vancouver area. A total of six different sausages were sampled from three vendors in the region. The six sausages were labelled by the vendors as: four *S. domesticus* (pork), one *B. taurus* (beef) and one *G. domesticus* (chicken). As the homogeneity of the sausages was unknown, three distinct locations on each sausage link were sampled (the middle and two ends of the sausage). DNA isolation, PCR and Gel electrophoresis were conducted on the samples. We found out that three pork-only sausages were labelled correctly. One sausage was labelled pork-only, but traces of *O. aries* (sheep) DNA were also found along with *S. domesticus* (pork). Results of the *G. domesticus* (chicken) and *B. taurus* (beef) sausage were unclear, and as such, no species could be concluded from the labelled sausages. Possible errors during the experiment caused beef and chicken to have such a result. However, the possibility of them containing species we did not test for also remains. Our experiment resulted in a lower-than-reported percentage of correctly reported meat in Canada. However, due to the small sample size, the experiment result can be insignificant. Thus, further studies on this topic are needed to yield a significant result. The result has shown that mislabelled meats are still available for sale in the Metro Vancouver area.

Introduction:

Mislabelling food products is a common problem in the global food industry. In Malaysia, a study has found that only 21 out of a sample containing 143 pre-packed meat products have been labelled correctly (1). Unexpected components, such as canine meat, can also be found in chicken nuggets in Malaysia (6). Undeclared components in sausage are also a common problem in Canada, with 10 out of 100 sausage samples from supermarkets containing undeclared species (7). Corporations and businesses have shown to be willing to mislabel their products to gain consumers' favour to increase sales, especially in the fish market (3).

With such knowledge in mind, we have decided to conduct a study on sausages in Metro Vancouver and see if the meat in the sausages is exactly what the vendors claimed. Sausages are one of Canada's most popular food items, and there is a variety of sausages in the market for consumption. It is important to get what you expect when purchasing meat products since certain types of meats are not consumable for many people due to diet, health and religious reasons.

Polymerase Chain Reaction (PCR) has been proven to identify meat products reliably with gel electrophoresis (2). For meat such as cattle, pig, goat, sheep and chicken, PCR could still amplify the DNA fragments even if the meat sample was cooked (5). This helps us have more freedom when choosing samples for the study, as we now can select a variety of sausage types like fresh (uncooked), smokies (slightly cooked) and lyoner (fully cooked).

According to the Global Food Safety Index 2022, Canada took the number one spot for the Food Safety and Quality category (8). We hypothesise that no harmful components will be found in the sausage meat, and at least 80% of the sausage meats will be labelled accurately with all types of meats. Our study will investigate whether sausage meats in the Metro Vancouver area are labelled correctly, with all their components declared as advertised.

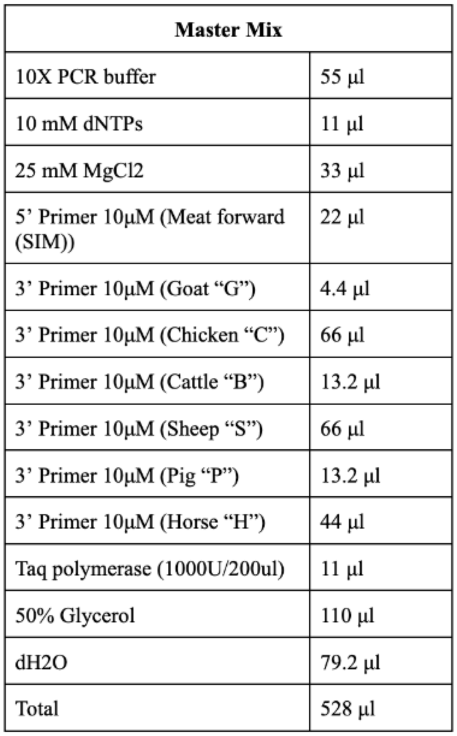
Methods:

Six sausage link samples from three different sources were collected with various labelled meats containing pork, cattle, and chicken. Ingredients for the sausage were documented based on the vendor's claims. Once the sausages were collected, they were preserved in a low-temperature environment (i.e., fridge, cooler) overnight before the experiment date. Since the homogeneity of the sausage meat was unknown, three different samples from different locations on the sausage (middle and two ends of the sausage) were collected to ensure the sausage was well represented. Eighteen samples were collected for the next step, DNA extraction.

A			B			C			D			E			F		
1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
Pork			Pork			Chicken			Pork			Pork			Beef		

Figure 1. The corresponding collected sausage samples and what species they claim to be in them. A letter was assigned to each sausage collected. Three different spots on each sausage were taken as samples with the numbers corresponding to (1 = top, 2 = middle, 3 = bottom).

DNA extraction started with small pinky-size sausage meat taken out from the three designated spots on each sausage (1 = top, 2 = middle, 3 = bottom). The samples were then placed into an Eppendorf tube and mashed with a sterile toothpick, followed by the addition of 300 uL of Cell Lysis Solution with Proteinase K. The tubes were then incubated in a 65ºC water bath for fifteen minutes to speed up the reaction. During the incubation period, the tubes were removed from the water bath to vortex for ten seconds for every five minutes interval. After that, the tubes were placed into an ice bath for five minutes. Once the tubes were cooled, 150 uL of Protein Precipitate Reagent was pipetted and vortexed for another ten seconds. The tubes were then transferred to the centrifuge machine and centrifuged for ten minutes at maximum speed (10000 rpm) to make the supernatant. After centrifugal, the supernatant solution was extracted to the new Eppendorf tube with 500 uL of cold isopropanol added. These new tubes would then be centrifuged at maximum speed for another ten minutes, and a small pellet should be formed and visible. The isopropanol was then removed and replaced by 500 uL of ethanol to rinse off any leftover salt. Repeated the ethanol rinsing once more before leaving the



Master Mix	
10X PCR buffer	55 µl
10 mM dNTPs	11 µl
25 mM MgCl2	33 µl
5’ Primer 10µM (Meat forward (SIM))	22 µl
3’ Primer 10µM (Goat “G”)	4.4 µl
3’ Primer 10µM (Chicken “C”)	66 µl
3’ Primer 10µM (Cattle “B”)	13.2 µl
3’ Primer 10µM (Sheep “S”)	66 µl
3’ Primer 10µM (Pig “P”)	13.2 µl
3’ Primer 10µM (Horse “H”)	44 µl
Taq polymerase (1000U/200ul)	11 µl
50% Glycerol	110 µl
dH2O	79.2 µl
Total	528 µl

Figure 2. PCR Master Mix Ratio

tubes upside down at room temperature with the cap open to dry out any remaining ethanol.

The next step was to perform PCR on the samples. 19 PCR reactions were needed, with 18 for the samples and one negative control. All DNA pellets were first resuspended in the TE buffer. Next, a Master Mix (MM) using the amounts in Figure 2 was made for our 22 samples. A total of six primers were used to identify six different species, including Goat, Chicken, Cattle, Sheep, Pig and Horse. For each PCR reaction, a volume of 24µL MM was added into each PCR tube, followed by 1.0µL of one sample DNA pellet + TE buffer solution. The negative control was a PCR tube containing 24µL of the MM and 1.0µL of distilled water to ensure the MM solution was not contaminated. Lastly, the PCR tubes were placed in the PCR machine and proceeded orderly with phases shown in Figure 3.

Phase 1	95°C 2 min
Phase 2	(95°C 30 sec, 60°C 30 sec, 72°C 30 sec) X 35 times
Phase 4	72°C 5 min.
Phase 5	4°C overnight
Phase 6	Stored in freezer

Figure 3. The PCR cycle is used for our isolated DNA.

The final procedure was Gel electrophoresis for species identification. First, 6 uL of 6X loading dye were pipetted to each of the 19 PCR samples in the PCR tubes. Then, the samples were mixed by pipetting up and down. Afterwards, 12 uL of the sample were loaded to the pipette tip and released into the gel. The process was repeated for each PCR sample. The samples were then run on a 3% gel (3g of agarose/100mL TAE buffer) at 50 volts for about 10 mins to get the sample out of the wells and then for about an hour at 120 volts. Finally, the gel was then viewed under UV for the result. Each lane of the gel was compared to the ladder to determine the amount of base-pair, and they were recorded for result analysis.

Results:

Six sources of sausages were experimented on; each source was sampled three times, resulting in a total of 19 samples, including a negative control. If the meats procured were correctly labelled, we would expect each triplet of samples A, B, D, and E to contain only pork, while samples C and F contain only chicken and beef, respectively.

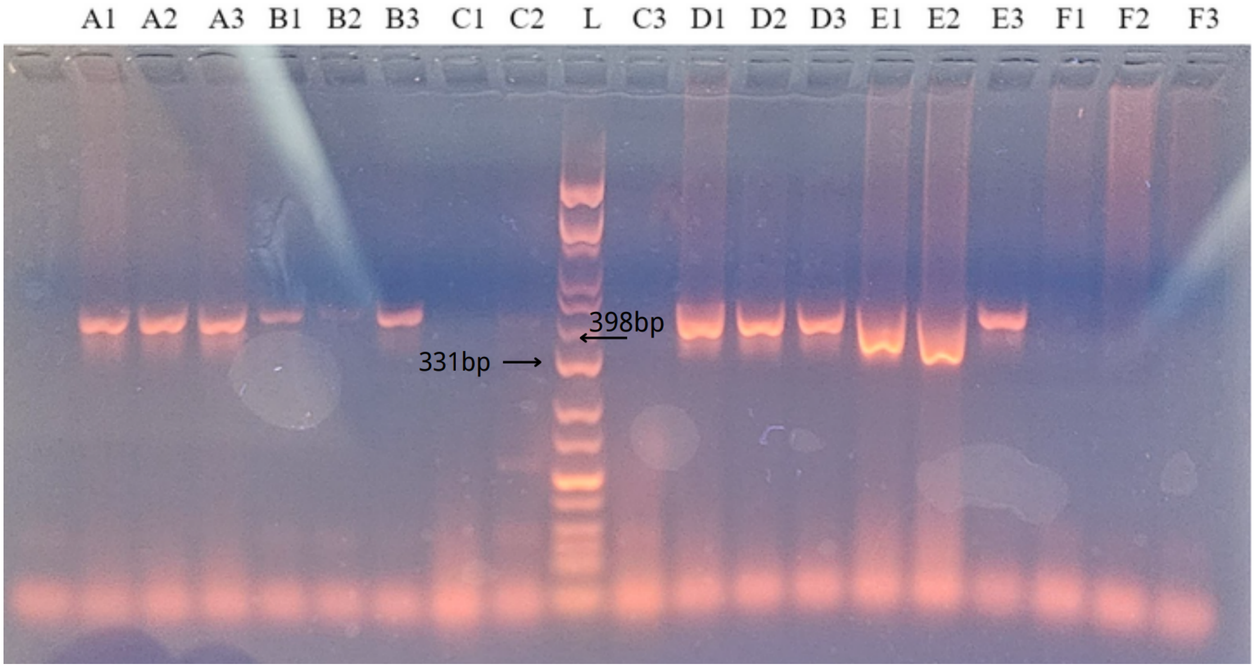


Figure 4. A picture after the gel electrophoresis for the 18 samples, one control, and one ladder was completed. (A, B, C, D, E, and F correspond to the six different sources collected - The gel below reads from left to right: Control, A1, A2, A3, B1, B2, B3, C1, C2, ladder, C3…F1, F2, F3). Along the ladder are species-specific DNA fragments of 157, 227, 274, 331, 398, and 439 bp from goat, chicken, cattle, sheep, pig and horse meats, respectively (5)

Our observational results from the figure above show that the triplet samples of A, B, and D contain only pork as the bands of each sample from the sources correspond to 398bp. Sources C and F show no bands across their gel arrays, so they are inconclusive. Finally, source E has two samples that show bands at 331 bp and one sample with a band at 398 bp corresponding to sheep and pigs, respectively.

Discussion:

The observed results from DNA isolation and gel electrophoresis provide valuable insight into the composition of sausage samples. We can observe the comparison of the DNA of the collected samples to specific sequences unique to different species being tested for and, as a result, the identification of the species found in the samples. It was observed that Sausages A, B, and D were correctly labelled and matched their designated contents. Sausage E was incorrectly labelled and found to contain a mix of sheep and pork. Sausages C and F is an open case with multiple potential conclusions. The samples may have contained species that were not tested for this experiment. Another conclusion could be that the sample contained a species being tested for this experiment; however, an experimental error was made. Some potential sources of error speculated by our team could be in the DNA extraction step. The DNA was denatured and therefore unable to be used for PCR; too little DNA was present for PCR to work effectively.

To clarify, the case of sources C and F showed no sources of the species that were being tested for. If we look at Figure 4, specifically at the bands C and F and compare them to the control, there are faint bands that the control does not have. This suggests that there may be DNA but not enough of a specific bp to produce meaningful results. PCR did not replicate what we needed to identify the specific species being tested. Therefore, either source C or F being a specific species is inconclusive. Given the time and resources, it would be recommended to replicate the experiment to observe a retrial of the results to see if there are any clarifications or possible variations that may occur.

Regarding Sausage E, other meat may have gotten into the sausage casing during production. Whether this is a mistake or not is unknown. Nevertheless, this issue should be communicated with the sausage producers. To confirm whether the mixing of meats occurred during the production process, the government should verify the accuracy of their labelling practices, investigate their production processes, and conduct independent tests on their products.

Moreover, another possibility exists that the sausage producer suppliers provided incorrect meat. If that is the case, collaboration with the suppliers to ensure the accuracy of the meat supplied is needed. Tests on their meat to confirm whether the mixing of meats occurred at the supply stage are highly recommended.

A collaboration between Erciyes University and Yeditepe University research group conducted a similar study to ours regarding DNA analysis. The research group focused on three sausage groups: horse, donkey, and pork. Opposed to our six different sausage meats of focus: goat, chicken, cattle, sheep, pig and horse meats. The research group conducted a similar DNA extraction and PCR test to what we did; they just amplified their results. They found 227 bp of pork, which matches ours (4). Also, at 153 bp of horse meat, surprisingly less than our 157 bp (4). This discrepancy between 153 bp and 157 bp is due to the research group also analyzing donkey meat, reaching 145 bp. They stated that it was very challenging to distinguish between donkey and horse meat due to them being closely related species (4). They didn’t elaborate further, but we can assume that the discrepancy is due to ion strength on the gel electrophoresis or some other factor (4).

Conclusion:

This study investigated six sausage samples, four of which were labelled as pork-only, one labelled as chicken-only, and the last one labelled as beef-only. Our mission is to verify the contents that manufacturers claim their products have. Using DNA isolation, PCR, and Gel electrophoresis, we found that three of the four pork-only sausages contained only the pork base pair length for the gel electrophoresis and thus were labelled correctly. One of the pork-only labelled sausages showed DNA with a base pair length of *O. aries* (sheep) and *S. domesticus* (pork). Our chicken-only results showed no clear bands, and no conclusion could be made about the species present in it. Our one beef-only sausage results showed no bands on the gel, and as such, no species could be concluded for what it was labelled.

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