DNA Barcoding of Meat for Species Identification

Kaitlyn Crawford
Professor Johnston

Abstract:
This investigation tested ground meat for horse DNA. Ouchterlony was performed to test for the presence of equine antigen. DNA extraction, PCR, and Gel Electrophoresis were carried out to prepare DNA for sequencing and DNA Barcoding. Ouchterlony results were questionable. The DNA sample from PCR was not of high enough quality to send out for sequencing. Results of this project were inconclusive.

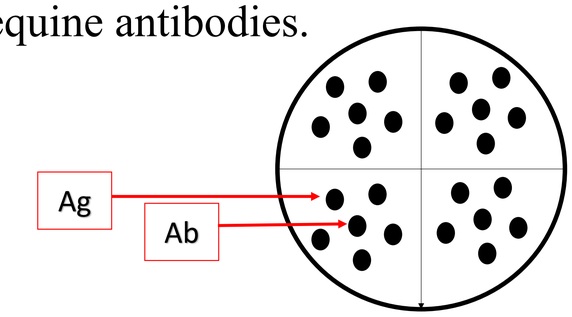
Introduction:

Cases involving horse meat contamination in ground meat marketed as beef has been reported. There have been scandals in the U.S. and U.K..[1] During a meat identification lab using ouchterlony for the Immunology class in Fall 2021, there were several results that tested positive for beef and horse. The two meats were saved, and an additional three different ground meats were purchased to compare; each from Giant food market.

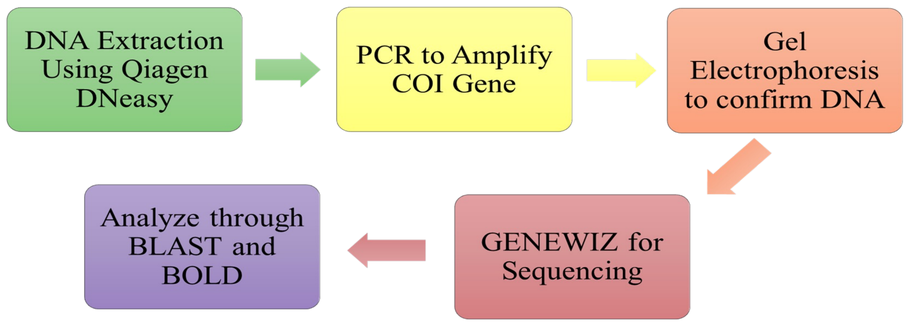
Method:

1. Samples: ground beef 1 (M1), ground beef 2 (M2), ground beef 3 (M3), ground beef 4 (M4), ground beef 5 (M5)

2. Ouchterlony – Blood was extracted from each meat sample and tested against anti-bovine and anti-equine antibodies.
   - 1% agar plates
   - Center well – antibody
   - Outer wells – antigen
   - Precipitin line – positive result



3. DNA Barcoding – Single Nucleotide Polymorphisms within highly conserved genes identify DNA at the species level



Results:

The ouchterlony showed a potentially positive result for equine DNA in M1 and M2 (Figs. 1, 2) while the beef had a potentially positive result in M4. The control plate was negative. DNA extraction was repeated twice due to error, as well as the PCR and Gel Electrophoresis. The initial attempt showed little to no DNA indicating the DNA extraction failed (Fig. 3). The second attempt lacked clear bands, indicating that the PCR may have failed (Fig. 4).

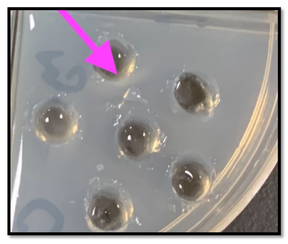


Fig. 1- Positive Control

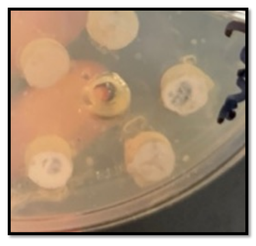


Fig. 2- Equine Plate Positive

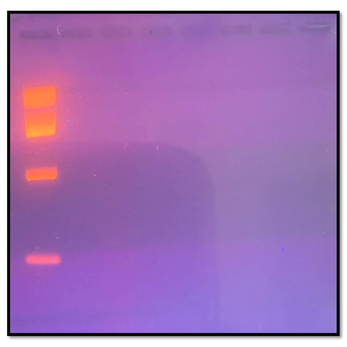


Fig. 3- DNA Ladder only

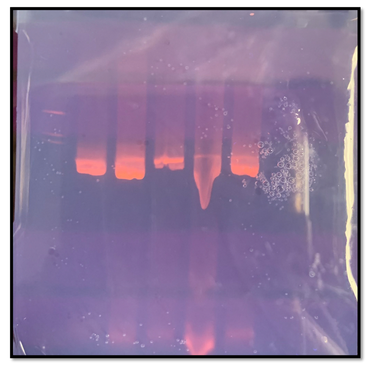


Fig. 4- Positive DNA

Discussion:

Although precipitin lines formed between meat extract and antibody, the lines were not as clear as the control. The results are inconclusive. The first DNA extraction was not successful. The second worked but the PCR was not successful. This was likely due to the amount of precision required for the procedure. The second round yielded DNA for each meat, but the bands were streaking. This means that DNA was extracted, but the PCR may not have worked. After consideration, it was determined that the technique used for micropipetting and therefore, the primer concentration was incorrect. Sterility and precise measuring is necessary when working with DNA. The next step would be to repeat the experiment to send out for barcoding.

Conclusion:

Without clear results from the ouchterlony, no conclusions can be drawn about the meat content of the ground beef. Additionally, the Barcoding could not be completed due to poor amplification. This approach could be optimized to improve DNA quality for sequencing.

References:

Thank you, Bristol Myers Squibb for making this possible and Professor Johnston, for the continuous support throughout the project.

1. “Horsemeat Scandal: The Essential Guide.” *The Guardian*, Guardian News and Media, 15 Feb. 2013, https://www.theguardian.com/uk/2013/feb/15/horsemeat-scandal-the-essential-guide.
2. “Ouchterlony Double Immunodiffusion.” *Ouchterlony Double Immunodiffusion - an Overview | ScienceDirect Topics*, https://www.sciencedirect.com/topics/immunology-and-microbiology/ouchterlony-double-immunodiffusion.
3. *Ouchterlony Immunodiffusion of Cell Wall Carbohydrate Extracts (Outer ...*https://www.researchgate.net/figure/Ouchterlony-immunodiffusion-of-cell-wall-carbohydrate-extracts-outer-wells-tested-with_fig1_12735187.
4. “Processed Beef Products and Horse Meat.” *GOV.UK*, https://www.gov.uk/government/news/processed-beef-products-and-horse-meat.