7-17-2016

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Recommended Citation

Perestam A.T., Fujisaki K.K., Nava O. & Hellberg R.S., Comparison of realtime PCR and ELISA-based methods for the detection of beef and pork in processed meat products, *Food Control* (2016), doi: 10.1016/j.foodcont.2016.07.017.

Accepted Manuscript

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PII: S0956-7135(16)30380-2

DOI: 10.1016/j.foodcont.2016.07.017

Reference: JFCO 5143

To appear in: *Food Control*

Received Date: 13 May 2016

Revised Date: 13 July 2016

Accepted Date: 15 July 2016

Please cite this article as: Perestam A.T., Fujisaki K.K., Nava O. & Hellberg R.S., Comparison of real-time PCR and ELISA-based methods for the detection of beef and pork in processed meat products, *Food Control* (2016), doi: 10.1016/j.foodcont.2016.07.017.

Comparison of Real-time PCR and ELISA-Based Methods for the Detection of Beef and Pork in Processed Meat Products

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Abstract

Two commonly used methodologies for species detection within processed meat products are real-time polymerase chain reaction (PCR), a DNA-based method, and enzyme-linked immunosorbent assay (ELISA), a protein-based method. In this study, a real-time PCR assay was compared to a commercial ELISA kit based on sensitivity, specificity, agreement among duplicate samples, cost, time, and ease of use. Fifteen reference samples containing known percentages (0.1-99.9%, w/w) of pork and beef were analyzed in duplicate using both methods. Thirty commercial products, including sausages, pet treats, and canned meats, were also tested in duplicate with each method. Reference sample analysis showed real-time PCR was able to detect pork in duplicate samples at 0.10% and beef at 0.50% in the binary mixtures. ELISA detected pork in duplicate samples at 10.0% and beef at 1.00% in the binary mixtures. When the results of reference and commercial samples were combined, real-time PCR demonstrated the greatest agreement among duplicate samples, at 96.7%, compared to 95.6% agreement for ELISA. The real-time PCR assay used in this study was found to be less expensive, while ELISA was less time-consuming and easier to perform. Both methods were successful at identifying species in ground meats, sausage, and deli meat samples; however, pet treats and canned meats proved more challenging. Overall, it was determined that the real-time PCR assay was optimal for species identification in processed meat products when a low detection limit is required; however, the ELISA kit may be advantageous in other situations due to its ease of use.

Keywords: Real-time PCR, ELISA, Mislabeling, Species Identification, Beef, Pork

1. Introduction

Meat and meat-based products make up a significant percentage of the American diet, with beef and pork being the top two red meats consumed (USDA 2013). On a per capita basis, consumption levels in the U.S. for 2016 have been projected to be 24.5 kg for beef and 21.3 kg for pork (USDA 2013). These species are also significant contributors to the global food trade, with 59.0 million tons of beef and veal and 109.3 million tons of pork expected to be produced globally in 2016 (USDA 2016). Meat species are often identifiable when sold as whole cuts; however, processing conditions and techniques may change the texture, flavor, and color of meat, making it difficult to authenticate species in food products containing processed meats (Cawthorn, Steinman, & Hoffman, 2013). The inability to readily identify meat species in processed products gives rise to the potential for species mislabeling, in which one species is substituted either partially or completely for another species.

In many cases, species mislabeling is a form of economically motivated adulteration (EMA), in which a product is intentionally mislabeled for reasons of economic gain (FDA 2009). Due to price differences among meat species, there are economic incentives associated with meat species mislabeling. For example, the average retail value of beef in 2015 was US$13.31/kg while the average retail value of pork for the same year was US$8.49 (USDA 2016), resulting in an economic incentive for the substitution of pork for beef in a processed product. Substitution can occur at any point in the supply chain, from the slaughterhouse up until the point of sale (Premanandh, 2013). Meat products may also become mislabeled due to cross contamination when processing different types of meat on the same equipment, as has been suggested in previous studies (Hsieh, Woodward, & Ho, 1995; Kane & Hellberg, 2016; Okuma & Hellberg, 2015).

Mislabeling of meat species in processed foods and pet foods has a number of potentially detrimental effects such as the exposure of consumers and pets to meat allergen risks, infringement of religious practices, and economic deception (Ballin, 2010). A number of studies have detected undeclared species in processed meat products such as deli meats, minced meats, burger patties, sausage, pet foods, and canned meats (Ayaz, Ayaz, & Erol, 2006; Cawthorn et al., 2013; Di Pinto et al., 2015; Flores-Munguia, Bermudez-Almada, & Vazquez-Moreno, 2000; Hsieh et al., 1995; Okuma & Hellberg, 2015). Some of the most commonly undeclared species within these products were beef, pork, poultry, and sheep. These findings are concerning from a religious point of view, as some religions prohibit the consumption of beef and/or pork (Sattar et al., 2004). In addition to the studies mentioned above, horsemeat was detected as an undeclared ingredient in numerous beef products in the 2013 horsegate scandal in Europe (National Audit Office 2013).

Adulteration and misbranding of meat products is prohibited under the United States Code (USC) Meat Inspection Act (21 USC § 610) (United States Code [USC] 2011). Adulteration and misbranding are considered to be separate terms, but both include forms of mislabeling. For example, adulteration can occur when an ingredient has been completely or partially omitted, and/or if any ingredient has been substituted within a meat product. A misbranded product includes one whose labeling is false or misleading or if it is offered for sale under the name of another food.

In order to determine if meat species have been partially or completely substituted for cheaper alternatives in processed food products, DNA or protein-based methods are often used (Ballin, 2010). Two of the most commonly used methods for this purpose are enzyme-linked immunosorbent assay (ELISA), a protein-based method, and real-time polymerase chain reaction

(PCR), a DNA-based assay. The USDA Food Safety and Inspection Service (FSIS) Microbiology Laboratory Guidebook relies on a sandwich ELISA method for identifying animal species in cooked and canned meat and poultry products (USDA 2005). Sandwich ELISA is recognized as being sensitive and robust and it has been used in several studies for detecting low levels of a target species within mixtures (Jones & Patterson, 1985; Liu, Chen, Dorsey, & Hsieh, 2006; Yamamoto, Kato, Endo, Kotoura, & Takeda, 2015). On the other hand, real-time PCR is a commonly used method for detection of meat species in processed products (Camma, Di Domenico, & Monaco, 2012; Kesmen, Gulluce, Sahin, & Yetim, 2009; Lahiff et al., 2002) and it has been used in a number of previous studies to reveal instances of product mislabeling (Kane & Hellberg, 2016; Okuma & Hellberg, 2015). A real-time PCR assay developed by Camma et al. (2012) was reported to demonstrate high sensitivity, specificity, and repeatability for meat species identification within meat mixtures. The assay was based on TaqMan™ chemistry with minor groove binder (MGB) probes and targeted regions of the cytochrome *b* and 16S rRNA genes. The authors reported the ability to identify turkey, chicken, beef, pork and sheep species in raw and cooked meat mixtures using this assay.

While both ELISA and real-time PCR are commonly used methods for species detection in foods, a direct comparison of the two methodologies for the purpose of detecting meat species in processed products has not been carried out. The results of such a comparison would help to facilitate the detection of food fraud by indicating which methodology is more appropriate for use with processed meat products. Therefore, the objective of this study was to compare the commercial ELISA kit described in the USDA/FSIS Microbiology Laboratory Guidebook to a published real-time PCR assay for the detection of beef and pork species in processed meat samples. Real-time PCR primers and probes developed by Camma et al. (2012) were chosen for use in this study because they have been extensively tested and validated for detection of the target meat species in both raw and cooked samples. The methods were evaluated on multiple factors, including sensitivity, specificity, agreement among duplicate samples, hands-on technician time required per sample, total time required per sample, cost per sample, and ease of use.

2. Materials and Methods

2.1. Reference sample collection and preparation

Lean, raw cuts of beef and pork were purchased from a local supermarket and were used in this study to create reference tissue mixtures. Prior to mixing, a 25 mg portion of raw meat from each species was collected to undergo DNA extraction and serve as a positive control for real-time PCR. The positive controls for ELISA were provided by the manufacturer. Reference tissue mixtures were prepared using 0.1-99.9% w/w of one meat species mixed with the second species, with a total weight of 50 g per sample (Table 1). Each meat sample was homogenized with 50 mL of sterile deionized water in a 12-speed Oster blender (Neosho, MO, USA) for 1 min at speed 6. Blender parts were cleaned and sterilized in between each sample. Blended samples were refrigerated overnight.

Next, blended samples were heat-treated following the USDA protocol (USDA 2005) for the identification of animal species in meat and poultry products, with some modifications. A portion (20 ± 2 g) of the blended sample was combined with 40 ± 0.5 mL of sterile deionized water in a sterile jar. The sample jar was covered with a screw-top sterile lid and placed in a 95 °C water bath for 15 ± 1 min, then cooled to room temperature. The contents were then poured into a sterile 24-oz Whirl-pak bag (Nasco, Salida, CA) and mixed in a Stomacher® 400 Circulator (Seward, Davie, FL, USA) for 60 s at 230 rpm. The bag was removed from the

Stomacher® and left undisturbed for 1 h at room temperature. Next, approximately 50 mg of tissue from each sample was transferred into two separate 1.5 ml Safe-Lock microcentrifuge tubes (Eppendorf, Hauppauge, NY, USA) and stored at -20 °C until DNA extraction. Two 1.5-mL aliquots of the liquid portion of the mixture were transferred to two separate 1.5 mL Safe-Lock microcentrifuge tubes and centrifuged at 10,300 rpm for 10 min. Next, 1.0 mL of each supernatant was transferred into a 1.5 mL Safe-Lock microcentrifuge tube and stored at -20 °C until ELISA.

2.2. Commercial sample collection and preparation

A total of 30 different commercial samples consisting of ground meats, cooked sausage, deli meats, pet treats, and canned meats were collected for beef and pork species identification using both real-time PCR and ELISA methods. A total of 11 samples listed beef or a beef derivative as an ingredient, 11 samples listed pork or a pork derivative as an ingredient, and 8 samples listed both species or their derivatives as ingredients. Each commercial meat sample was prepared for DNA extraction and ELISA as described in section 2.1, with the exceptions that the cooked meat samples were not heat-treated and that 60 ± 0.5 mL of sterile deionized water was added to 20 ± 2 g of the pet treat samples prior to use of the Stomacher to allow for improved mixing of the low-moisture products.

2.3. DNA extraction and quantification

DNA extraction was performed for all reference and commercial samples in duplicate using the DNeasy Blood and Tissue Kit, Purification of Total DNA from Animal Tissues, Spin-Column Protocol (Qiagen, Valencia, CA, USA), according to the manufacturer's instructions. Lysis was carried out at 56 ˚C for 1-3 h or until tissue was completely lysed, with vortexing every 30 min. DNA was eluted in 50 µL of pre-warmed (37 °C for 30 min) Buffer AE. The extracted DNA was then quantified using a NanoDrop 2000 (Thermo Scientific, Waltham, MA, USA). Samples were diluted with AE Buffer to normalize the DNA concentration to 250 pg/µL for use in real-time PCR. Samples with less than 250 pg/µL after DNA extraction were not diluted. All DNA samples were stored at -20 ˚C until real-time PCR. A reagent blank with no sample added was included with each set of DNA extractions.

2.4. Real-time PCR

Real-time PCR was carried out on all duplicate DNA samples using a Rotor-Gene® Q Thermal Cycler (Qiagen) combined with species-specific primers and TaqMan™ probes developed previously for detection of pork and beef (Camma et al., 2012). Reactions were carried out in a singleplex format and targeted 166-bp (pork) and 183-bp (beef) regions of the gene coding for cytochrome *b.* Primers were synthesized by Integrated DNA Technologies (Coralville, IA, USA) and probes were obtained from Applied Biosystems (Austin, TX, USA). Each reaction tube contained 10 µL of TaqMan Fast Universal PCR Master Mix (2X) (Applied Biosystems), 2.0 µL of TaqMan MGB probe (2.5 µM), 2.0 µL of each oligonucleotide primer (3.0 µM for beef, 9.0 µM for pork), 2.0 µL of molecular-grade water, and 2.0 µL of DNA or negative control for a total reaction volume of 20.0 µL. A non-template control (NTC) containing water instead of DNA was included alongside each set of samples tested with real-time PCR. Each PCR run also included three positive controls of the target meat species diluted to 250 pg/µL, 25 pg/µL, and 2.5 pg/µL. Cycling condtions were followed as described in Camma et al. (2012): 95 ºC for 20 s, followed by 35 cycles of 95 ºC for 1 s and 60 ºC for 20 s. The results of each run were analyzed with the Rotor-Gene Q Software v.2.2.3 and a sample was determined to be positive if it had a Ct value for the meat species being tested (Okuma & Hellberg, 2015). Results obtained were qualitative and reported as presence or absence of the target species.

2.5. ELISA

ELISA was carried out for all samples in duplicate using the supernatants prepared as described in section 2.1. Each duplicate sample was tested with both the ELISA-TEK Cooked Beef Species Kit and the ELISA-TEK Cooked Pork Species Kit (ELISA Technologies, Gainesville, FL, USA) following the USDA protocol for identification of animal species in meat products (USDA 2005). A Thermo Scientific AccuWash microplate washer was used to complete all wash steps. Each test run included four positive controls and four negative controls supplied by the kit. The results were read with a BMG Labtech FLUOstar Omega microplate reader (Cary, NC, USA). Samples were determined to be if positive if the raw OD value of one or both of the replicate wells was greater than 0.250 and if the results of all controls were in accordance with USDA guidelines (USDA 2005).

3. Results and Discussion

3.1. Specificity and sensitivity

As shown in Table 1, both ELISA and real-time PCR showed 100% specificity during reference sample testing, with no cross-reactivity detected for the non-target species in the pork/beef binary mixtures. In terms of sensitivity, ELISA was able to consistently detect pork in the binary mixture at levels down to 10.0% w/w (Table 1; Sample 5). Although pork was detected at levels as low as 5.00% w/w (Sample 4), this result was only found with one of the duplicate samples. The beef-specific ELISA test showed greater sensitivity compared to the pork-specific test, with the lowest detection at 0.50% w/w (Sample 12) and the lowest consistent detection level at 1.00% w/w (Sample 11) for beef within a binary mixture. In comparison, the USDA lists adulteration detection limits of 4% w/w for pork and 1% w/w for beef in binary mixtures of sample extracts when using the ELISA-TEK™ Cooked Meat Species Test Kits, with the caveat that the sensitivity and specificity of each assay may vary depending on the lot that is being tested (USDA 2005). It is possible that the assay was less sensitive for pork in the current study due to differences in preparation methods for the binary mixtures. However, the USDA protocol does not provide details on this point, making it difficult to elaborate further. Interestingly, the USDA protocol is based on a sandwich ELISA with polyclonal antibodies (pAbs); however, previous studies using a sandwich ELISA assay with monoclonal antibodies (mAbs) have reported lower detection limits for both pork and beef. For example, studies using a sandwich ELISA with porcine-specific mAbs have reported the ability to detect pork at levels of 0.05-0.5% w/w in various meat mixtures (Chen & Hsieh, 2000; Liu et al., 2006). Similarly, Yamamoto et al. (2015) were able to detect beef at levels of 0.1% w/w in a beef and pork meal mixture using a sandwich ELISA with two bovine-specific mAbs. Although the goal of the current study was to compare the USDA protocol for ELISA to a real-time PCR assay, future research along these lines should include consideration of ELISA with mAbs.

As shown in Table 1, the real-time PCR assay showed greater sensitivity as compared to the commercial ELISA kit for both the beef and pork assays. The lowest consistent level of detection for pork within the binary mixture was 0.10% w/w using real-time PCR (Sample 1), as compared to 10.0% with ELISA. This was the lowest percentage of pork contained in a reference sample, meaning that the assay may be capable of even greater sensitivity. Similarly, Laube et al. (2003) were able to detect pork species at 0.1% w/w in a beef/pork mixture by applying real-time PCR with TaqMan^{TM} probes. Rodriguez et al. (2005) also showed detection of pork at the lowest level tested (0.5% w/w) in cooked pork/beef mixtures using real-time PCR with TaqMan^{TM} MGB probes. In the case of beef, the lowest level consistently detected with real-time PCR in the current study was 0.50% w/w within the binary mixture (Sample 12), as compared to 1.00% for ELISA. This detection level was also found in a previous study utilizing a real-time PCR assay with TaqMan^{TM} probes for the detection of meat species in mixtures Dooley et al. (2004). On the other hand, Laube et al. (2003) showed slightly greater sensitivity for beef detection at 0.1% w/w in a beef/pork mixture tested with real-time PCR.

3.2. Commercial samples

The ability of ELISA and real-time PCR to detect pork and beef in commercial samples was tested with a variety of processed meat products, including ground meat, sausage, deli meat, pet treats, and canned meat (Table 2). Overall, the two methods showed agreement as to the species detected in the products for 26 out of the 30 samples. Among the 26 samples showing agreement between the two methods, 23 of these were found to be correctly labeled and 3 were potentially mislabeled (Samples 34, 38, and 40). Among the four samples with inconsistent results between the two methods, two were found to be correctly labeled by at least one method (Samples 37 and 39) and two were found to be potentially mislabeled (Samples 44-45). These samples are discussed in detail in the following paragraphs.

Both ELISA and real-time PCR were able to positively identify pork in 16 of the 20 products (80%) that specifically listed pork or a pork-derived ingredient on the label. One of the pet treats (Sample 38) and two canned meats (Samples 44-45) were negative for pork with both ELISA and real-time PCR. The pet treat listed bacon fat as the twelfth ingredient in the product and the two canned meats each listed pork as the fifth ingredient in the product. The inability of ELISA to detect pork in bacon fat is not surprising, given that ELISA is a protein-based assay; however, real-time PCR would have been expected to detect DNA in this product, based on previous studies involving pet foods (Okuma & Hellberg, 2015). Species detection in the canned meat samples may have been limited due to the extensive processing that these products undergo as well as the presence of inhibitory ingredients present in these samples, or these could represent instances of mislabeling. Because neither method was able to detect the target species in these three products, they were determined to be potentially mislabeled.

There were two pet treats (Samples 37 and 39) for which only one of the two methods was able to detect pork. Sample 37 tested positive for pork with ELISA but negative with real-time PCR, even though it listed pork as the first ingredient on the label. This result may have been due to the presence of numerous plant-based ingredients in the sample, which are known to have compounds that are inhibitory to PCR (Schrader, Schielke, Ellerbroek, & Johne, 2012). On the other hand, Sample 39 tested positive for pork with real-time PCR and negative with ELISA. This sample listed bacon seventh in the ingredient list and may have contained pork below the level of detection for ELISA. Interestingly, one of the pet treats (Sample 34) tested positive for pork with both ELISA and real-time PCR even though pork was not specifically stated on the label. This product listed animal liver flavor as an ingredient, which was likely the source of the detected pork.

Of the 19 commercial samples that declared beef or a beef-derived ingredient on the label, ELISA identified beef in 14 products (74%) and real-time PCR identified beef in 16 of the products (84%). Two pet treats (Samples 34 and 38) and one canned meat product (Sample 40) tested negative for beef with both methods. Similar to the results discussed above involving bacon fat, one of the pet treats (Sample 34) listed beef tallow, a form of beef fat, as the only beef ingredient. The other pet treat that tested negative for beef (Sample 38) listed beef as the eighth ingredient on the package and the canned meat product (Sample 40) that tested negative for beef was a corned beef hash that listed beef as the first ingredient. These three products were determined to be potentially mislabeled due to the negative test result with both real-time PCR and ELISA. Two additional canned meats (Samples 44-45) tested positive for beef with real-time PCR, but negative with ELISA. These samples listed beef as the second and fourth ingredient, respectively.

When comparing the five categories of commercial products tested, the ground meat samples were the most identifiable by ELISA testing. The sausage and deli meat samples only showed one inconsistent ELISA result each between the two products. For real-time PCR, the ground meat, sausage, and deli meat samples were all identifiable and showed consistent results among duplicate samples. The pet treat and canned meat products also showed a high level of consistency among duplicate samples for both ELISA and real-time PCR, with only one inconsistent result found for one of the samples tested with real-time PCR. However, there were several instances in which a declared species in these product types was not detected by one or both methods. This was likely due to a number of factors, including high heat treatment, the presence of inhibitory ingredients, an inability to identify species in animal-derived fats, and mislabeling of the products. The findings indicate that in some cases, it may be beneficial to use a combination of real-time PCR and ELISA in order to more accurately authenticate the product label. For example, when detecting species within pet food products, real-time PCR would be advantageous for the detection of meat species at low levels, while ELISA may help to overcome false negatives due to PCR inhibition.

3.3. Agreement among duplicate samples

Of the 15 reference samples, the pork-specific ELISA showed agreement among duplicates for all but one sample, which contained 5.00% pork and 95.0% beef (Sample 4; Table 1). The beef-specific ELISA assay also had one reference sample that did not show agreement among duplicates, which contained 99.5% pork and 0.50% beef (Sample 12). Both samples were just below the lowest consistent level of detection for the assay. Of the 30 commercial products, the pork-specific ELISA showed 100% agreement among duplicates and the beef-specific ELISA showed agreement for all but two samples (Table 2; Samples 26 and 32). In total, ELISA showed agreement among duplicate samples for 86 out of 90 tests performed, with the pork-specific assay showing greater agreement (97.8%) compared to the beef-specific assay (93.3%) (Table 3).

As compared to ELISA, real-time PCR showed slightly greater overall agreement among duplicate samples, with 100% agreement for all reference samples and 95% agreement for commercial samples (Tables 1-2). Of the 30 commercial products, two canned meats (Samples 44-45) showed inconsistent results when attempting to detect beef using real-time PCR and a pet treat product (Sample 36) showed inconsistent results for pork detection (Table 2). Overall, real-time PCR analysis showed agreement among duplicate samples in 87 out of 90 tests performed, with the pork-specific assay demonstrating higher agreement (97.8%) than the beef-specific assay (95.6%) (Table 3).

3.4. Time requirements and ease of use

The commercial ELISA kit was found to be easier to carry out and have shorter time requirements as compared to the real-time PCR assay (Table 3). The hands-on technician time required to complete the ELISA test was approximately 2 min faster per sample and 1.9 h faster per 24 samples as compared to real-time PCR (Table 3). Both assays involved the use of multichannel and/or electronic pipets, which improved ease of use and reduced the hands-on and total time required.

Overall, ELISA was found to be easy to moderate to carry out. The main technical challenge of ELISA was found to be timing the addition of the Stop Solution in order to achieve the absorbance values for the positive controls called for in the USDA protocol (USDA 2005). The real-time PCR assay was considered to be moderately difficult to perform. The main technical challenge of this assay was the need to determine the DNA concentration for each sample and perform dilutions prior to performing PCR. This is especially tedious when large numbers of samples are being analyzed.

The total assay time required for ELISA was about 3.3 h for one sample and about 3.8 h for 24 samples (Table 3). These values include the time required for processing the four positive controls, four negative controls and two blanks, which adds approximately 9 min to the total assay time. The total assay time does not include sample preparation and collection of the supernatant, which is dependent on the nature of the sample and can range from 20 min per sample for cooked products to 30 min per sample for uncooked products. The times observed in the current study are similar to those reported by the manufacturer of the ELISA-TEK kits: about 30 min of hands-on time for sample preparation and collection of the supernatant, followed by 3 h to conduct a complete ELISA test (ELISA Technologies Inc. 2016).

The total assay time required for real-time PCR was about 3.4 h for one sample and about 5.6 h for 24 samples (Table 3). The total assay time includes the use of the three positive controls, a reagent blank, and a no-template control, which adds approximately 10 min. Total assay time for sample preparation and collection of tissue is not included. Unlike ELISA, uncooked samples do not require heat treatment prior to analysis with PCR. Therefore, the time required for sample preparation and collection of tissue is estimated to take an additional 20 min per sample.

3.5. Cost of assay

The sample costs associated with real-time PCR were found to be less expensive than the sample costs associated with ELISA. The 2016 list price for a 96-well, ELISA-TEK™ Cooked Meat Specification Kit was US$550, resulting in a cost per well of US$5.73. However, this price does not factor in the positive controls, negative controls, or blanks, which all together occupy an additional eight wells and add a cost of US$46 to each ELISA run. Since each run must include controls and blanks, the maximum value will be obtained by running a full plate of samples rather than testing a few samples at a time. For example, when including the costs of the controls, the price would be US$52 to test one sample at a time, but would be reduced to US$7.64 per sample if 24 samples were run simultaneously (Table 3).

The real-time PCR beef and pork assay used in this study was determined to cost US$4.49 per sample tube. These costs include the 2016 list prices of a DNeasy Extraction Kit (50 reactions), beef and pork TaqMan MGB probes, beef and pork forward and reverse primers, and 2X TaqMan Fast Universal PCR Master Mix (250 reactions). As with the ELISA cost calculations, these costs do not factor in the use of positive and negative controls, which must be included with each run. The controls included in this study for each assay were the reagent blank from DNA extraction, three positive DNA controls for PCR, and a non-template PCR control, which would add a total cost of US$9.57 to each run.When the controls are included in the calculation, the total cost becomes US$14 to test one sample at a time and US$4.89 per sample when testing 24 samples simultaneously (Table 3).

4. Conclusions

Overall, the results show that the real-time PCR assay used in this study was a more sensitive method for pork and beef species detection within ground meat products as compared to the ELISA protocol described by the USDA. However, due to the potentially inhibitory ingredients that are found within some processed meat products, it may be beneficial to use both real-time PCR and ELISA for species detection when testing products with additional ingredients, such as pet foods. The results of this study also suggest that the real-time PCR assay was a more reliable and less expensive method to perform when compared to the ELISA protocol. On the other hand, ELISA was found to be less time consuming and easier to perform than real-time PCR. It is important to note that the findings of this study are based on specific protocols, and other real-time PCR and ELISA protocols may show different results. To further compare real-time PCR and ELISA methodologies, it is suggested that sandwich ELISA assays with sensitivity limits closer to that of real-time PCR methods be used.

Acknowledgments

This project was funded in part by a grant from the National Science Foundation, Division of Earth Sciences, NSF-EAR #1359500. The authors would like to thank Chapman University's Office of the Chancellor for additional funding support. None of these entities were involved with the design or execution of the study.

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Table 1. Results of meat species identification testing in cooked porcine and bovine reference sample mixtures. The results of real-time PCR and ELISA are reported as positive (+) or negative (-) for each duplicate sample

Sample no.	**% Pork**[a]	**% Beef**	**Real-time PCR results**		**ELISA results**	
			Pork	**Beef**	**Pork**	**Beef**
1	0.10	99.9	+ / +	+ / +	- / -	+ / +
2	0.50	99.5	+ / +	+ / +	- / -	+ / +
3	1.00	99.0	+ / +	+ / +	- / -	+ / +
4	5.00	95.0	+ / +	+ / +	- / +	+ / +
5	10.0	90.0	+ / +	+ / +	+ / +	+ / +
6	25.0	75.0	+ / +	+ / +	+ / +	+ / +
7	50.0	50.0	+ / +	+ / +	+ / +	+ / +
8	75.0	25.0	+ / +	+ / +	+ / +	+ / +
9	90.0	10.0	+ / +	+ / +	+ / +	+ / +
10	95.0	5.00	+ / +	+ / +	+ / +	+ / +
11	99.0	1.00	+ / +	+ / +	+ / +	+ / +
12	99.5	0.50	+ / +	+ / +	+ / +	- / +
13	99.9	0.10	+ / +	- / -	+ / +	- / -
14	100	0	+ / +	- / -	+ / +	- / -
15	0	100	- / -	+ / +	- / -	+ / +

[a]Total weight of each sample was 50 g

Table 2. Results of meat species identification in porcine and bovine commercial samples using real-time PCR and ELISA. The results of real-time PCR and ELISA are reported as positive (+) or negative (-) for each duplicate sample

Sample No.	Product type	Meat ingredients on label	Real-time PCR results		ELISA results	
			Pork	Beef	Pork	Beef
16	Ground meat	Beef	- / -	+ / +	- / -	+ / +
17	Ground meat	Beef	- / -	+ / +	- / -	+ / +
18	Ground meat	Beef	- / -	+ / +	- / -	+ / +
19	Ground meat	Pork	+ / +	- / -	+ / +	- / -
20	Ground meat	Pork	+ / +	- / -	+ / +	- / -
21	Ground meat	Pork	+ / +	- / -	+ / +	- / -
22	Sausage	Beef	- / -	+ / +	- / -	+ / +
23	Sausage	Beef	- / -	+ / +	- / -	+ / +
24	Sausage	Pork	+ / +	- / -	+ / +	- / -
25	Sausage	Pork	+ / +	- / -	+ / +	- / -
26	Sausage	Pork Beef Turkey	+ / +	+ / +	+ / +	- / +
27	Sausage	Pork Beef	+ / +	+ / +	+ / +	+ / +
28	Deli meat	Beef	- / -	+ / +	- / -	+ / +
29	Deli meat	Beef	- / -	+ / +	- / -	+ / +
30	Deli meat	Pork	+ / +	- / -	+ / +	- / -
31	Deli meat	Chicken Pork	+ / +	- / -	+ / +	- / -
32	Deli meat	Pork Beef	+ / +	+ / +	+ / +	+ / -
33	Deli meat	Chicken Beef Hearts Pork	+ / +	+ / +	+ / +	+ / +
34	Pet treats	Chicken meat Beef tallow Animal liver flavor Salmon	+ / +	- / - [a]	+ / +	- / - [a]

35	Pet treats	Fish Beef lung Beef liver	- / -	+ / +	- / -	+ / +
36	Pet treats	Beef Pork liver Bacon	- / +	- / -	+ / +	- / -
37	Pet treats	Chicken fat Pork	- / -[a]	- / -	+ / +	- / -
38	Pet treats	Beef Chicken by-product meal Liver	- / -[a]	- / -[a]	- / -[a]	- / -[a]
39	Pet treats	Bacon fat Beef Bacon	+ / +	+ / +	- / -[a]	+ / +
40	Canned meat (corned beef hash)	Beef	- / -	- / -[a]	- / -	- / -[a]
41	Canned meat (roast beef)	Beef	- / -	+ / +	- / -	+ / +
42	Canned meat (pork)	Pork with ham Chicken	+ / +	- / -	+ / +	- / -
43	Canned meat (shredded pork meat)	Pork	+ / +	- / -	+ / +	- / -
44	Canned meat (chili)	Beef Pork	- / -[a]	- / +	- / -[a]	- / -[a]
45	Canned meat (Vienna sausage)	Chicken Beef Pork	- / -[a]	- / +	- / -[a]	- / -[a]

[a]Meat species listed on the product label could not be detected

Table 3. Comparison of real-time PCR and ELISA testing for the detection of pork and beef in mixed samples, based on observations from the current study. Time and cost calculations include the use of positive and negative controls

Characteristics	**Real-time PCR**[a]	**ELISA**
Sensitivity[b]	0.10% Pork; 0.50% Beef	10.0% Pork; 1.00% Beef
Specificity with reference samples	100%	100%
Agreement among duplicate samples[c]	97.8% Pork; 95.6% Beef	97.8% Pork; 93.3% Beef
Hands on technician time (per sample; per 24 samples)	0.4 h; 2.6 h	0.3 h; 0.7 h
Total time required (per sample; per 24 samples)	3.4 h; 5.6 h	3.3 h; 3.8 h
Cost (per sample; per 24 samples)	US$14; $117	US$52; $183
Ease of use	Moderate	Easy-moderate

[a]Including DNA extraction
[b]Lowest consistent detection level (w/w) in a binary mixture of pork and beef.
[c]Percentages are based on a total of 45 samples tested in duplicate with each assay

Highlights

- Real-time PCR detected beef consistently at 0.50%, compared to 1.00% for ELISA.
- Real-time PCR detected pork consistently at 0.10%, compared to 10.0% for ELISA.
- Compared to ELISA, real-time PCR showed greater agreement among duplicate samples.
- ELISA was found to be less time consuming and easier to perform than real-time PCR.
- ELISA and real-time PCR showed 100% specificity during reference sample testing.