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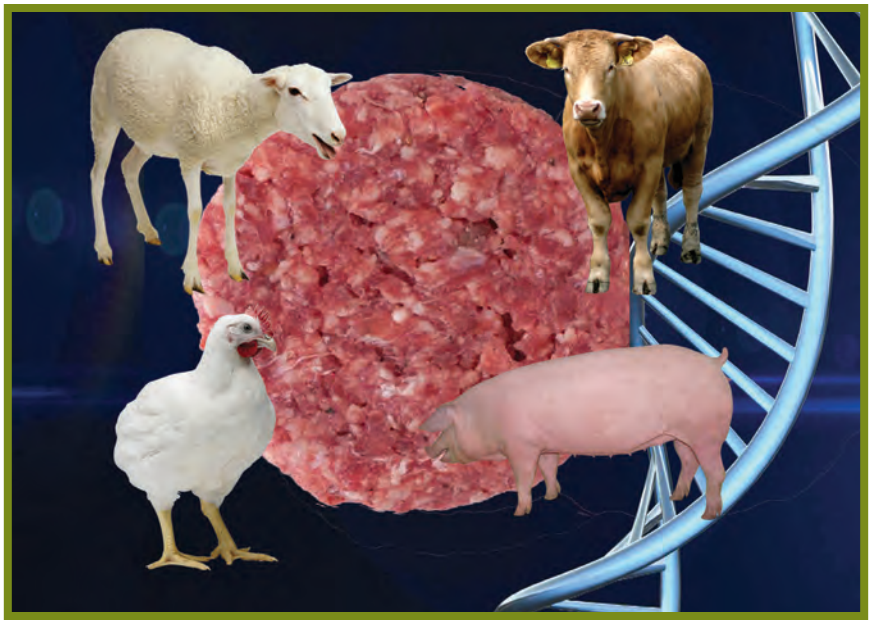

Undeclared Meat Species in Processed Meat Products from Retail Franchises in the Durban Metropole, KwaZulu-Natal Province, South Africa, Using Species-specific DNA Primers

ABSTRACT

This study investigated the authenticity of labelling and contents of meat products from selected retail franchises, using mitochondrial species-specific DNA primers. Sixty percent of mutton sausages and 100% of chicken sausages contained undeclared beef meat. Additionally, 33.3% of beef sausages were contaminated with pork and chicken meat, while 12.5% of beef patties were contaminated with pork meat. Of the beef sausages screened, 33% contained chicken meat. It was further observed that 25% of beef products did not contain beef or the other three meat species that were screened for. Of the interviewed retailers, 37.5% (3/8) acknowledged use of the same processing machinery for different meat species, 37.5% (3/8) used separate machinery for each meat type, and the remaining 25% used either separate or the same machinery, depending on the circumstances. Escalating meat prices and a decrease in economically sustainable meat production have been reported to contribute to the mislabelling and adulteration of meat and its by-products. However, contamination/adulteration of meat products detected in this study was most likely unintentional. The use of the same machine for processing different meat species without proper cleaning was most likely to have been the cause of contamination.

INTRODUCTION

Consumption of meat and meat products has escalated in recent decades, as these foods provide numerous nutrients that are valuable to humans *(9, 15, 24)*.

Statistically, meat and meat products are some of the most high-priced food products and are in high demand in many developing countries, including South Africa *(9)*. These include beef, mutton, pork and chicken, which are the most commonly consumed meats in South Africa *(9, 22)*. However, prices and demand for the meat products differ, based on the species or type of the animal *(9, 11, 18)*. For example, meat from highly valuable animal species are in high demand and more expensive than those from less valuable animals *(9, 11)*. Prices of beef and mutton and their by-products have increased dramatically since 2000 compared to prices of other meats such as chicken and pork, because of rising costs of production *(9)*.

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As the world population rises, the demand for meat products increases *(9, 37)*. Increases of meat prices, meat availability, meat quality, and nutritional value alter the selection of meat products by consumers *(26, 32)*. Such changes have been accompanied by increasing cases of mislabelling and adulteration/replacement of more expensive meat with cheaper meat or addition of a cheaper or less valued meat type to meet the growing demand and maximize profit margins *(1, 2, 25)*.

Meat mislabelling and adulteration in processed food/meat products can be intentional or unintentional *(7, 9)*. Cases of intentional meat mislabelling usually consist of substitution or addition of animal meat (normally cheaper) that is not declared in the ingredients list of the product *(5, 9, 35)*. In contrast, unintentional mislabelling/adulteration may be due to cross-contamination, which usually occurs when equipment used to process more than one meat species is not properly cleaned *(7, 9, 15)*.

According to Di Pinto et al. *(13)*, meat mislabelling or adulteration can have a negative impact on the consumer's health, through the consumption of products containing certain undeclared meat species or allergens that can prompt an allergic reaction *(15, 16, 28)*. For example, Ayuso et al. *(4)* reported an allergy prevalence of 73%, 58%, and 41% in beef, pork and chicken among 57 patients suspected of having meat allergies in the U.S. Furthermore, not declaring certain meat species violates the rights of individuals with certain religious beliefs that include dietary restrictions *(21, 27, 37)*; members of some religious groups are prohibited from consuming certain meat species, such as pork *(21, 27)*. As a result, food description or labelling must be truthful and accurate *(27, 30)*.

Against this background, several reliable and rapid methods to identify animal species in processed products have been developed *(10, 12, 31)*. Such methods include PCR with the use of species-specific primers, mtDNA, PCR-RFLP, DNA barcoding and PCR-RAPD. According to Dai et al. *(12)*, the PCR assay, based on mitochondrial species-specific primers, has been demonstrated to be a cheaper and less time-consuming technique for detection of meat mislabeling and adulteration in processed meat products compared with other PCR-based methods. The method does not require additional sequencing or further digestion of the PCR products with restriction enzymes/endonuclease *(17)*. Therefore, the aim of this study was to determine the extent of mislabelling and adulteration of meat products in selected retail franchises in the Durban metropolitan area, using PCR-based on species-specific primers, and to determine the level of knowledge and awareness of meat mislabelling/adulteration by the retailers.

MATERIALS AND METHODS

Study area and sample collection

Durban metropolitan is one of the largest cities in the KwaZulu-Natal province of South Africa, with a population of over 3.4 million. It is comprised of various ethnic groups; 51% of the population are Black Africans, 25.1% are Indians or other Asians, 15.3% are White and 8.6% are designated as Colored. As a result, consumption of meat types and their products is determined by the cultural/religious practices of numerous ethnic groups.

Six retail franchises (A, B, C, D, E and F), representative of the major retail supermarkets in Durban metropolitan, were randomly selected. From each franchise, one retail outlet was randomly selected, with the exception of franchise A and B, from which two retail outlets, separated by location, were randomly selected. A total of 40 processed meat products (20 burger patties and 20 sausages) from different animal species – cattle (beef), sheep (mutton), pig (pork) and chicken – were purchased from the selected retail outlets. Samples were comprised of sausages and burger patties either made from the retail outlet butchery or supplied already processed by the common suppliers of the franchise. All butcheries of the selected retail outlets of franchises used commercial sausage and burger patty making machines to process their meat products. Each sample represented a batch of a minimum of 10 sausages or burger patties of the same brand from which sub-samples were collected *(Fig. 1)*. Selection of meat products from each retail franchise was based on the different brand names supplied by the common suppliers, as well as the different meat products made by the retail butcheries of the franchises. To note is that retail outlets of the same or different franchises sourced their meat/meat products from the same suppliers and hence in our selection, meat products that were common to all franchises were selected only once, to avoid repetition of the same products. We ended up with 40 products, which could have been more if the same products were repeatedly selected.

DNA extraction

The processed meat products selected for sampling were rinsed two times with 70% ethyl alcohol and three times with distilled H_2O to reduce the concentration of oil/spices prior to sampling as described by Dai et al. *(12)*. An amount weighing 0.25 grams was sampled from each batch of sausages/patties, and DNA was extracted from each meat product, using DNeasy Tissue Mini-Prep Kit (Zymo Research Cooperation) (catalogue No D3051), according to the manufacturer's instructions. The DNA concentration and purity were checked by use of a spectrophotometer (NanoDrop), and the DNA was then used for PCR analysis.

PCR amplification and electrophoresis

A PCR assay was used to validate the meat types as previously described, and to check the presence of cross-contamination against the product and ingredient classification, using mitochondrial species-specific markers. Unprocessed mutton, beef, chicken and pork meat were used as positive controls, and water was used as a negative control.

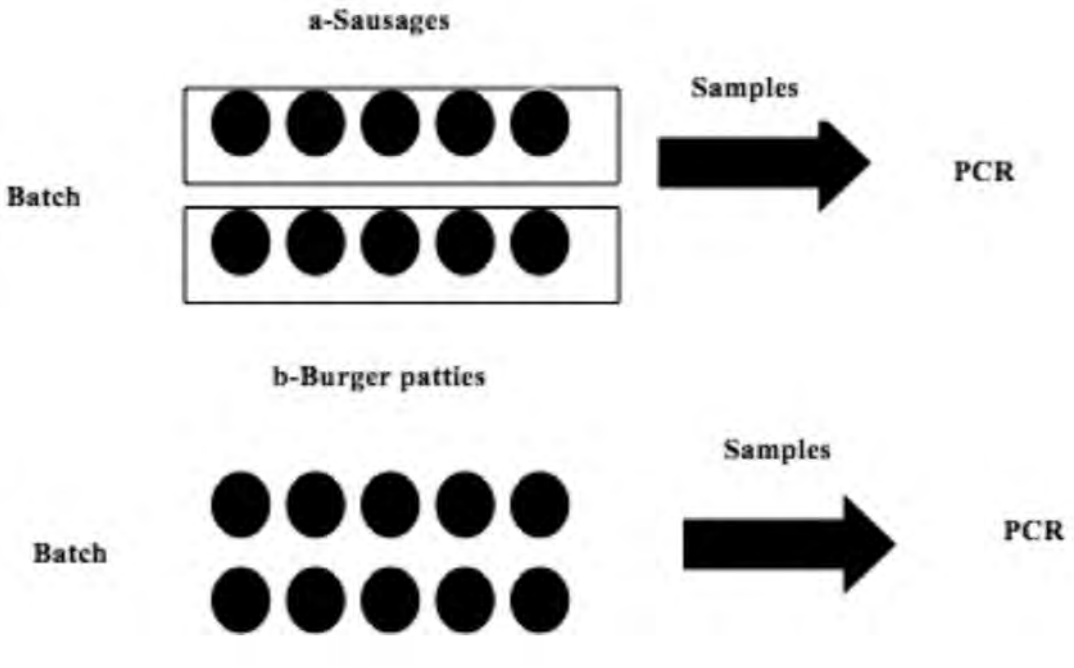

Figure 1. A batch was composed of 10 sausages or burger patties represented by a circle. From each batch, a subsample was taken to constitute a sample for PCR analysis.

TABLE 1. Meat adulteration/contamination of beef, mutton, pork and chicken products collected from 8 retail outlets representing 6 franchises in the Durban metropolitan

Meat type/ product	Number of retail outlets sampled	No. of samples collected	Animal species contaminant (% contamination)		
			Mutton	Chicken	Pork
Beef					
Sausages	5	6	0	(2 of 6) 33.3	(2 of 6) 33.3
Patties	6	8	0	(1 of 8) 12.5	(1 of 8) 12.5
Mutton			Beef	Chicken	Pork
Sausages	5	5	(3 of 5) 60	0	(1 of 5) 20
Patties	1	1	0	0	0
Chicken			Beef	Mutton	Pork
Sausages	2	2	(2 of 2) 100	(1 of 2) 50	0
Patties	6	11	(2 of 11) 18.2	(1 of 11) 9	(2 of 11) 18.2
Pork			Beef	Mutton	Chicken
Sausages	4	7	(2 of 7) 28.6	(2 of 7) 28.6	(2 of 7) 28.6

PCR amplification was performed in a 25 µL mixture containing 5 µL of genomic DNA extracted from each meat product, 1 µL of each primer, 12.5 µL Master mix and 5.5 µL of water. Thermal cycling (BIORAD) was performed under the following conditions: 5 minutes initial denaturation at 94°C; 40 cycles of denaturation at 94°C for 1 minute; annealing for 1 min at 69°C for chicken, 60°C for pork; extension at 72°C for 1 minute, and final extension at 72°C for 6 minutes *(15)*. A separate multiplex PCR was performed in a 25 µL reaction volume containing 2 µL of genomic DNA from each meat product, 2 µL of each forward primer of sheep and cattle, 4.5 µL of common reverse primer, 10 µL Top Taq master mix and 4.5 µL of water. A PCR was performed in a thermocycler machine (BIORAD) under the following conditions: 10 minutes initial denaturation at 94°C; 40 cycles of denaturation at 94°C for 1 minute; annealing for 1 min at 60°C; extension at 72°C for 1 minute, and final extension at 72°C for 7 minutes. A volume of 5 µl of each amplicon was electrophoresed in 2% suspension agarose gel stained with 100 µl Ethidium Bromide (0.5 mg/ml), at 80V for 60 minutes. A Uvitec UV transilluminator was used to visualize the DNA bands, and the image was captured using an Uvitec digital camera, with the positive sample identified by the pair size indicated in *Fig. 2*.

Sequencing

Amplicons of undeclared meat samples were randomly selected and sent for sequencing by Sanger dideoxy at Inqaba Biotechnical Industries (Pty.) Ltd., South Africa, for animal species authentication. DNA fragments were sequenced in the forward and reverse directions, using the same primers as in the initial amplification. Sequencing was then conducted, using the ABI V3.1 Big dye kit in accordance with the manufacturer's instructions and with use of ABI 377 automated sequencer. The Zymo Seq clean-up kit was used to clean the labelled products thereafter, and the products were injected into the ABI 3500XL Genetic Analyzer (with a 50 cm array), using POP7. The resultant sequences were manually edited using BioEdit v7.2.5 as described by Hall *(19)*. Identities of the sequences were confirmed by the Basic Local Alignment Search Tool (BLAST) of the NCBI (National Centre for Biotechnology; www.ncbi.nlm.nih.gov/).

Questionnaire

A questionnaire was administered through an interview to managers of the eight retail outlets from which the meat species/products were bought, in order to gather information on knowledge and awareness on meat adulteration/mislabelling.

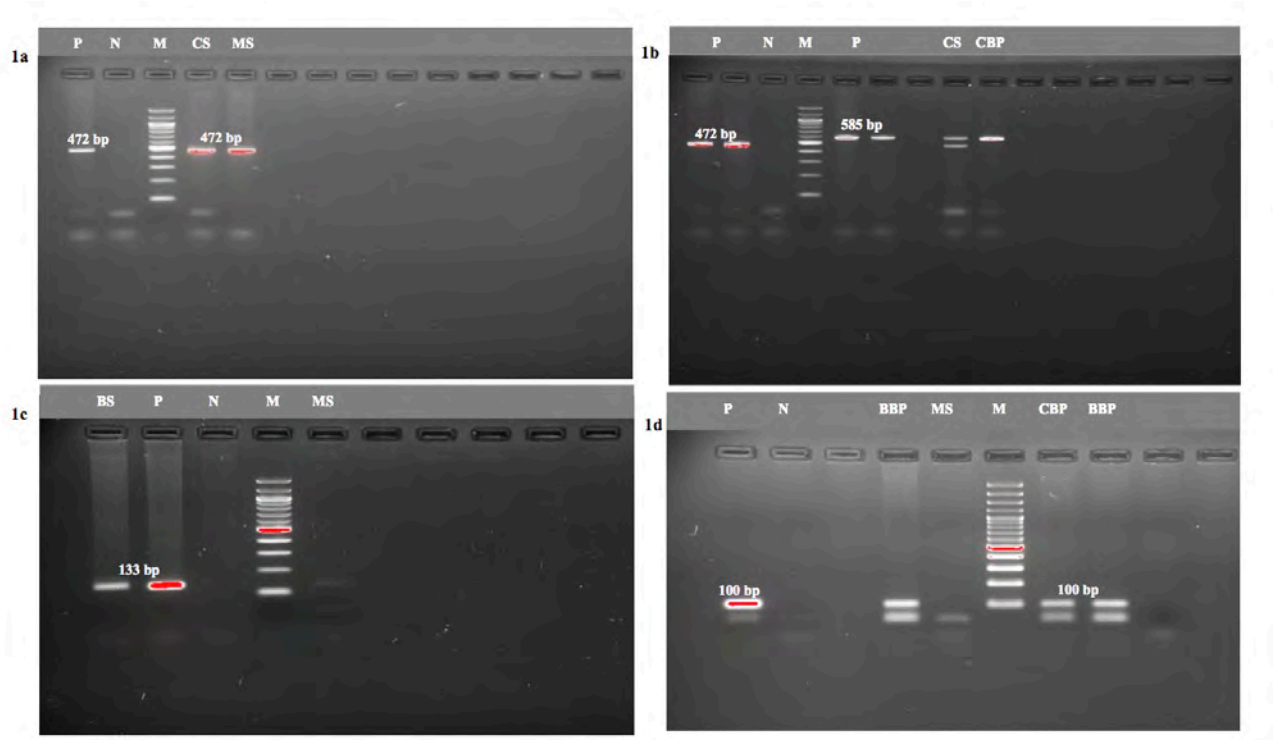

Figure 2. 2% Agarose gel electrophoretic analysis. Images 1(a) represents sausages that tested positive for beef meat, 1(b) represents the presence of undeclared beef and mutton meat in sausages and burger patties, 1(c) represent beef sausages that tested positive for undeclared chicken, 1(d) shows sausages and burger patties that tested positive/negative for pork. N = negative control, P = positive control, M = molecular weight marker, CS = chicken sausages, MS = mutton sausages, CBP = chicken burger patties, BS = beef sausages, BBP = beef burger patties.

Ethical considerations

The study was approved by the University of KwaZulu-Natal Animal Research Ethics Committee (Reference number: AREC/024/016M). Retail managers agreed to sign an informed consent form to participate in the study after the purpose of the study was explained to them by the first author. No personal information of the retail managers was recorded on the questionnaires.

Statistical analysis

Results were categorized and summarized in tables and figures. Prevalence of meat contamination was calculated using the following formula:

$$(P)\% = \frac{\text{Total no. of samples contaminated by a specific meat type undeclared}}{\text{Total no. of samples examined for the specific meat types as declared}} \times 100$$

Data from the questionnaire survey was summarized to assess the level of knowledge and awareness of meat mislabelling/adulteration by retailers and was further analyzed using SPSS (cross tab), where likelihood ratio and chi-square were used to assess whether there was an association between the different variables from the questionnaire.

RESULTS

Overall, of the 40 processed meat products sampled, 26 (65%) tested positive for meat adulteration, i.e., they contained meat species not declared on the product's ingredients list. Of the 26 contaminated products, beef *(Bos taurus)* was the leading contaminant, followed by pork *(Sus scrofa)*, chicken *(Gallus gallus)* and mutton *(Ovis aries)*. Sequence analysis and BLAST further confirmed contamination by classifying these contaminant species as follows: *Bos taurus* (beef), *Sus scrofa* (pork), *Gallus gallus* (chicken) and *Ovis aries* (mutton). In addition, there was a 99% sequence similarity for *Ovis aries*, *Bos taurus* and a 98% sequence similarity for *Sus scrofa* and *Gallus gallus*.

Beef sausages

Undeclared pork and chicken were both detected in 33.3% (2 of 6) screened beef sausages *(Table 1)*. Chicken was the main undeclared species in beef sausages purchased in 2 franchises (E and F) *(Table 2)*. No contamination was found in beef sausages purchased from either of the retail outlets of franchise A, whereas for franchise B, meat contamination by undeclared pork was observed in retail outlet 1 *(Table 2)*. *Figure 2* shows the agarose gel electrophoretic analysis profiles of meat types in which undeclared meat types were detected by use of species-specific DNA primers.

Beef patties

Undeclared pork and chicken were detected in 12.5% (1 of 8) and 12.5% (1 of 8) of beef burger patties, respectively *(Table 1)*. Beef DNA and DNA of the other investigated meat types were not detected in 25% (2 of 8) of declared beef burger patties *(Table 1)*. Beef burger patties in franchise B

TABLE 2. Results for beef products from 6 franchises represented by 7 retail outlets from the Durban metropolitan screened for undeclared mutton, chicken and pork

Retail franchises	Retail outlets	Product type	No. of sample batches	Undeclared animal species		
				Mutton	Chicken	Pork
A	1	BS	1	-	-	-
		BP	2	-	-	-
B	1	BS	1	-	-	+
		BP	1	-	-	+
	2	BS	1	-	-	-
		BP	1	-	-	-
C	1	BS	1	-	-	-
		BP	2	-	-	+
D	1	BP	2	-	+	-
E	1	BS	1	-	+	-
F	1	BS	1	-	+	-

BS = Beef sausages, BP = Beef burger patties

(Retail outlet 1) and C were contaminated by pork, while in franchise D, they were contaminated by undeclared chicken *(Table 2)*.

Mutton sausages

Beef was the major undeclared meat detected in 60% (3 of 5) of sausages declared as mutton *(Table 1)*. The presence of undeclared pork was found in 20% (1 of 5) of mutton sausages *(Table 1)*. Beef was detected in mutton sausages only in those sampled from franchises A (Retail outlet 1), C and E, and undeclared pork was detected only in mutton sausages from franchise B (Retail outlet 1) *(Table 3)*. In all retail outlets from the six franchises, no mutton sausages contained undeclared chicken *(Table 3)*.

Mutton patties

No undeclared meat species (0%) were detected in mutton burger patties purchased from franchise B (Retail outlet 2) *(Table 3)*.

Chicken sausages

Of the purchased chicken sausages, 100% (2 of 2) contained undeclared beef, and 50% (1 of 2) contained undeclared mutton *(Table 1)*. In chicken sausages purchased from franchise B (Retail outlet 2) and F, beef was the most common undeclared meat type *(Table 4)*.

Chicken patties

Pork was the most commonly undeclared species in chicken burger patties, followed by beef and mutton. The study detected 18.2% (2 of 11) contamination with undeclared pork, 18.2% (2 of 11) with undeclared beef and 9% (1 of 11) with undeclared mutton *(Table 1)*. Chicken burger patties from franchises B (Retail outlet (1) and C were contaminated with undeclared pork, and chicken burger patties from franchise A (Retail outlet (1) contained undeclared beef *(Table 4)*. In addition, undeclared mutton was observed only in chicken burger patties purchased from franchise B (Retail outlet 2) *(Table 4)*.

Pork sausages

In pork sausages, undeclared beef, mutton and chicken were detected in 28.6% (2 of 7), 28.6% (2 of 7), and 28.6% (2 of 7), respectively *(Table 1)*. Pork sausages from franchises C and D contained beef DNA *(Table 5)*. Also, pork sausages from franchises A (Retail outlet 1) and D were contaminated with mutton. Pork sausages from franchise D contained undeclared mutton, beef and chicken *(Table 5)*.

Questionnaire results

Eight retail outlets associated with the six franchises had never had complaints from their clients with regard to meat contamination or adulteration prior to this study, according to the managers (personal communication) *(Table 6)*. Furthermore, 37.5% (3 of 8) of retailers claimed to use one processing machine, which is cleaned after each meat species during meat product processing, 37.5% (3 of 8) claimed to use a separate machine for each meat type, and 25% (2 of 8) responded that, depending on circumstances, they used one processing machine, which is cleaned after each meat species during meat product processing, and sometimes use separate machinery for each meat type. There was no statistically significant difference observed between the nature of the retail butchery (halaal/non halaal) and where they purchased their meat (beef, mutton and chicken) ($P > 0.05$). However, the relationship between the nature of the retail butchery and where they purchased pork was significant ($P < 0.05$). Addi-

TABLE 3. Results for mutton products from 4 franchises represented by 5 retail outlets from the Durban metropolitan screened for undeclared beef, chicken and pork

Retail franchises	Retail outlets	Product type	No. of sample batches	Undeclared animal species		
				Beef	Chicken	Pork
A	1	MS	1	+	-	-
B	1	MS	1	-	-	+
	2	MS	1	-	-	-
		MP	1	-	-	-
C	1	MS	1	+	-	-
F	1	MS	1	+	-	-

MS = Mutton sausages, MP = Mutton burger patties

TABLE 4. Results for chicken products from 6 franchises represented by 8 retail outlets from the Durban metropolitan screened for undeclared beef, mutton and pork

Retail franchises	Retail outlets	Product type	No. of sample batches	Undeclared animal species		
				Beef	Mutton	Pork
A	1	CP	3	+	-	-
	2	CP	3	-	-	-
B	1	CP	1	-	-	+
	2	CS	1	+	+	-
		CP	1	-	+	-
C	1	CP	1	-	-	+
D	1	CP	1	-	-	-
E	1	CP	1	-	-	-
F	1	CS	1	+	-	-

CS = Chicken sausages, CP = Chicken burger patties

TABLE 5. Results for pork products from 4 franchises represented by 4 retail outlets from the Durban metropolitan screened for undeclared beef, mutton and chicken

Retail franchises	Retail outlet location	Product type	No. of sample batches	Contaminating animal species		
				Beef	Mutton	Chicken
A	1	PS	3	-	+	-
B	1	PS	1	-	-	-
C	1	PS	1	+	-	-
D	1	PS	2	+	+	+

PS = Pork sausages

tionally, the relationship of the nature of the retail butchery to measures taken to avoid meat adulteration in retail butchery was not significant ($P > 0.05$).

Product ingredients listing

Of 40 meat products purchased, 2.5% (1 of 40) was classified only as chicken burger patties, without presenting the ingredients list. It was further observed that 25% (2 of 8) of beef products did not contain beef or the other three meat species, which were screened against. Additionally, 5% (2 of 40) had inconsistencies in their ingredient listing; for instance, although the ingredients list stated that the meat product contained chicken and/or turkey, one might not know whether the product contained both chicken and turkey, or either turkey or chicken.

DISCUSSION

This study demonstrated that of the 40 processed meat samples examined, 65% were contaminated by other meat types that were not declared. Contamination was more common in sausages than in patties; 80% of the sausages were contaminated with one or more meat types, compared with 50% of burger patties. Results also indicated that of the four meat types (mutton, beef, chicken, pork), beef

TABLE 6. Retail managers' responses on survey of knowledge and awareness of meat contamination/adulteration in 8 randomly selected retail outlets representing 6 franchises in the Durban metropolitan

Questions	Responses	Percentage (%)
Category of staff interviewed	(1) Butchery manager	100
	(2) Other	0
Methods/processes that are used to avoid meat contamination/adulteration	(1) Separate machinery	37.5
	(2) One machine cleaned after each meat species	37.5
	(3) Both 1 and 2	25
Complaints from clients regarding meat contamination	(1) Yes	0
	(2) No	100
Awareness of meat adulteration	(1) Yes	50
	(2) No	50
Measures taken to avoid meat adulteration	(1) Carcass of the same meat species hang together, different meat types are cut and packaged separately	50
	(2) Did not know	50
Awareness of any legislation regarding meat adulteration	(1) Yes	50
	(2) No	50
If no, how would you like to be educated in this matter?	(1) Educate employees	62.5
	(2) Would like further educational materials through email	12.5

was the most frequent contaminant in products labelled as other species. This is unexpected, because beef has a higher market price than chicken and pork. However, Hsieh et al. *(23)* reported that beef and mutton are commonly found to be contaminating species in ground meat in retail outlets, and this might also be the case in our study. The other reason for substituting more expensive meat such as beef and mutton for cheaper meat such as poultry may be because of use of unmarketable trimmings from expensive meats *(15, 23)*. Alternatively, the contamination could be due to the repeated use of the same grinding machine or processing equipment for different meat species without proper cleaning in between *(3, 15, 23)*. Similarly, questionnaire results indicated the repeated use of the same processing equipment for more than one meat type by some retailers. Furthermore, other authors have indicated that the presence of undeclared beef could be due to non-fat powder milk, often added to processed meat products to increase the overall yield and improve the taste of the product *(7, 14)*. Similarly, in this study, the presence of undeclared beef in processed meat products was most likely due to addition of non-fat powdered milk that was not declared on the product label. Furthermore, 25% of beef products deemed beef did not contain either beef, mutton, chicken or pork DNA, which led to the conclusion that the meat belongs to other meat species that we could not determine.

According to Barakat et al. *(6)*, pork is the most commonly used species to adulterate high-value meat species such as beef and lamb. Similar findings were observed in this study, in which a high proportion of beef and mutton products were found to be contaminated with pork. Ballin *(5)* reported that often animal meat or fats from one species is replaced by fats from other species. However, in the case of low-valued meat or fats such as pork, this practice may be argued to be intentional *(7)*. This malicious practice may result in a huge effect on individual health and conflict with religious beliefs *(6)*, for people allergic to the contaminants as well as to members of some religious sects for whom consuming pork is against their dietary laws *(33, 36)*.

The study also showed that chicken was the third contaminating meat species, following pork, which was more common in beef meat products, along with pork. According to Surowiec et al. *(38)*, the high amounts of undeclared chicken and pork in processed meat products such as burger patties and sausages could be due to the use of mechanically recovered meat (MRM), which is commonly produced from pork and chicken carcasses *(6, 9)*. Surowiec et al. *(38)* further explained that these ingredients (paste-like) are usually added as a cheap source of proteins in comminuted meat products such as burger patties, sausages and deli meats. Though use of MRM may be a common practice with chicken and pork,

use of this technique in products such as boerewors, species sausage and mixed species sausages is illegal in many countries, including South Africa *(9)*. As such, failure to disclose the use of MRM in the ingredients list of a product not only violates the labelling regulations but also put the lives of individuals with allergy at risk *(6)*. Additionally, in this study, in all products screened there was no declaration of use of MRM on the ingredients list. This lack of consistency and transparency is against the South African food regulatory bodies, which has published regulations covering clear declaration of all food constituents and ingredients on product labels, including common allergens *(9)*.

In order to effectively monitor meat adulteration and mislabelling in processed meat products, reliable molecular analytical tests, continuous species monitoring and stricter regulations are required to enforce correct species declaration on processed meat products *(7, 15, 29)*. Use of acceptable levels/dosage of certain ingredients (e.g., for individuals with allergies), the use of tolerable and specific ingredients, and proper cleaning of equipment should be practiced to avoid contamination *(9, 31, 34)*. Additionally, meat products processors and retail butcheries must be aware of the possible risks of unwanted ingredients such as allergens and meat cross-contamination during the processing of food products *(7, 31, 34)*. Taken together, these will reduce the rate of meat adulteration and mislabelling in processed meat products *(7, 14)*. Although the new advanced techniques, including PCR with use of species-specific primers, are able to identify meat species in processed products, quantitative estimations need to be considered to determine the extent of adulteration and whether meat adulteration was accidental or intentional *(8)*. Therefore, further research to develop easy and inexpensive methods is needed *(13, 20)*.

CONCLUSION

Results showed the presence of undeclared meat types in processed meat products in major meat retail franchises in the Durban metropole. It was not possible to determine whether the contamination was intentional, for economic gain, or was a result of cross-contamination. To be certain whether the contamination was intentional, it is important to determine the proportion or quantity of undeclared meat type. Few studies have investigated mislabelling and adulteration of meat products in South Africa, including KwaZulu-Natal province, and more studies focusing on quantifying the level of contamination and the extent of mislabelling meat products should be conducted and should include both wildlife and domestic processed meat products.

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