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Original Research Article

DNA-Based Identification of the Origin of Red Meat Products: A Case Study of Sucuk in Ödemiş, İzmir, Türkiye

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Abstract: Determining the species origin of meat and meat products is important for protecting consumers. Molecular methods such as polymerase chain reaction (PCR) are powerful techniques developed to detect and quantify adulterants in meat species. In this study, it was aimed to determine the origins of three red meat products (sucuk) purchased from butchers in Ödemiş (İzmir, Türkiye) on August 13, 2024, using the TaqMan q-PCR method. DNA isolation of the samples was performed using the Eurofins GeneSpin DNA Isolation Kit (Freiburg, Germany). The research samples were analyzed separately for species origin concerning cattle, sheep, and chicken using species-specific primer and probe sets from the same company and the TaqMan q-PCR method. As a result of the study, it was determined that the examined meat products were of cattle and sheep origin. However, all samples tested negative for chicken-specific analyses. The Cycle threshold (Ct) mean values for cattle and sheep species were found to be 13.25 and 23.50, respectively. The study results confirmed that the species origins of the meat products in Ödemiş (İzmir, Turkey) were consistent with the information stated on their labels. Additionally, within the scope of protecting public health, it is recommended that local authorities conduct broader-scale research and inspections on businesses engaged in meat production.

Keywords: DNA, Ödemiş, Red meat, Sucuk, TaqMan q-PCR.

INTRODUCTION

Meat is one of the food products with high nutritional value. The high price of meat and its decreasing availability over time have led some meat producers and sellers to engage in counterfeiting of meat and meat products. One of the most common economic frauds in the meat industry is the substitution of more expensive meat with cheaper or lower quality meat (Kumar *et al*., 2015).

Consumers must be protected from fraudulent meat adulteration practices due to various reasons such as economic losses, health effects, and religious beliefs. Simple, accurate, precise, and sensitive methods are needed to detect meat fraud and identify meat species (Meyer *et al.,* 1994).

A variety of traditional methods are used for the identification of meat species, including anatomical, histological, biochemical, spectrophotometric, chromatographic, immunological, and enzyme-linked immunosorbent assays (ELISA). Some of these methods are inadequate for species identification in meat products that have been exposed to high cooking temperatures (Guoli *et al*., 1999).

In recent years, DNA-based methods, such as Polymerase Chain Reaction (PCR) techniques developed based on molecular approaches, have provided better identification solutions that can overcome the limitations of other methods. DNA analysis methods based on polymerase chain reaction (PCR) can accurately and rapidly detect animal species used in meat products, even those subjected to intensive heat treatment and having a complex composition (Chikuni *et al*., 1994).

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In this study, the origin of three red meat products (sucuk) obtained from butcher shops in Ödemiş (İzmir) will be investigated using the TaqMan q-PCR method for cattle, sheep, and chicken species. The obtained results will be compared with the origin information declared on the labels of the sucuk products to determine whether there is any mislabeling or adulteration. The research findings will provide up-to-date and reliable information on the origin of sucuk sold in Ödemiş.

MATERIALS AND METHODS

Three red meat products, sausages, were purchased from butchers at the public market in Ödemiş (İzmir) district. The meat products were transported to the laboratory under a cold chain environment and stored at +4°C until the research process (Figure 1).

DNA Extraction and Determination of DNA Concentration

Approximately 350 g of meat product from each sausage sample was ground using a commercial blender to obtain a homogeneous paste. A 0.2 g sample of the homogenized sausage paste was transferred into a 2 mL Eppendorf tube. DNA isolation of the meat products was performed using the Eurofins GeneSpin DNA Isolation Kit, following the manufacturer's protocol recommendations (Anonymous 2024).

The concentrations of approximately 200 mg DNA extracts isolated from meat products, sausages were measured at 260 nm using a NanoDrop 2000 spectrophotometer (Thermo Scientific). The DNA concentration was adjusted to 40 ng/µL by dilution. The A260/A280 ratio was considered to verify the purity of the samples (Kim *et al*., 2016).

Determination of Target Gene and Primers

The target gene studied was the mitochondrial cytochrome b (cytb) region, while the 18S rRNA conserved region was used as a reference. Pure reference DNAs of cattle, sheep, and chicken species, along with primers and probes labeled with 5'-carboxyfluorescein (FAM) and 3'-fluorescent quencher (HEX) fluorescent dyes, were obtained from Sia Analiz (Izmir, Türkiye) and Analytik-Jena (Jena, Germany) (Anonymous 2024).

TaqMan qPCR Analysis

The study utilized the real-time TaqMan qPCR method. TaqMan qPCR reactions were prepared in a total volume of 20 µL, consisting of 40 ng of genomic DNA and 3 µM of cattle, sheep, and chicken primers and probes (Table 1).

The TaqMan qPCR process was carried out on Rotor Q (Qiagen) and (Eppendorf, Mastercycler Realplex2) devices over 35 cycles and was evaluated using the red, yellow, turquoise, brown, olive green, and dark green channels (Table 2).

Specificityc and Sensitivity Tests of Primer and Probe Sets

In the study, primer and probe sets specifically designed for cattle, sheep, and chicken species by Analytik-Jena were used. For sensitivity determination, the DNA isolates of the samples were diluted with water, adjusting the DNA concentration to 0.1 ng/5µL. A total of 2 µL of template DNA was added to each reaction tube. Within the scope of the TaqMan probe method, reaction analyses of the species-specific primer-probe sets with dilution-characterized samples were performed. A standard curve was created using the cycle threshold (Ct) values corresponding to the DNA concentrations of the diluted samples (Anonymous 2024).

Species Determination with TaqMan qPCR Technique

For the determination of the species origin of meat products in sucuk as research material, commercial test kits Innu DETECT Beef, Sheep, Chicken Assay Kit (Analytik-Jena, Germany), containing species-specific primers for cattle, sheep, and chicken, as well as Taq-Polymerase, were used. This study aimed to determine the species origin of meat products in sucuk using these commercial test kits and the TaqMan qPCR technique for cattle, sheep, and chicken (Anonymous 2024).

RESULTS

In this study, three red meat products, sucuk, were obtained from butcher shops in Ödemiş (İzmir, Türkiye). It was stated that the sucuk samples were produced from beef and mutton. As part of the preliminary assessment, a taste and odor analysis was conducted on the sucuk samples, and it was predicted that these products were made from beef and mutton. Subsequently, a quantitative method was chosen to fully and accurately identify the origin of the sucuk samples. For this purpose, the species origin of the meat products in the sucuk samples was determined using the TaqMan q-PCR method. Within the scope of this method, 350 g of samples were taken from each sucuk for DNA isolation. DNA isolation from the meat mixtures of the samples was performed using the commercial Eurofins GeneSpin DNA Isolation Kit (Germany).

Determination of Ct Value with TaqMan qPCR

The cycle threshold (Ct) value, also known as the threshold cycle, refers to the number of cycles required for the fluorescent signal generated by species-specific primers and probes in the TaqMan qPCR method to exceed the background signal. The Ct value is used to determine the presence and relative abundance of the target product in the test material.

A sensitivity value has been determined for defining the densities of DNA isolates from the samples. For this purpose, water dilutions at a level of 0.1 ng/5µL were prepared from the DNA isolations of the samples. Analytical studies were conducted based on the cycle threshold (Ct) values corresponding to the 0.1 ng/5µL DNA concentrations of the diluted samples.

During the analysis process, three red meat products were examined separately for cattle, sheep, and chicken species using species-specific primer and probe sets and the TaqMan qPCR method. As a result of the analysis, the Ct values of the meat products were determined. In all samples, the Ct values for cattle and sheep species yielded positive results. The average Ct values for cattle and sheep species were calculated as 13.25 and 23.50, respectively. Based on these data, it was determined that the proportion of beef was higher in the examined sausage products. In contrast, the Ct analyses specific to chicken resulted in negative outcomes for all samples. Thus, it was confirmed that there was no presence of chicken meat in the analyzed meat products (Table 4.1). The qPCR results of the samples are shown in red, yellow, turquoise, brown, olive green, and dark green. The analyses were performed in duplicate (Figures 2-4).

Species Detection with TaqMan qPCR

As part of the research, dilution series at a 0.1% level were prepared using 10 ng of DNA obtained from sucuk products, which were claimed to be made from beef and mutton, collected from butcher shops in the Ödemiş district (İzmir). For species identification of the meat products, a two-biological-replicate analysis was conducted using the TaqMan qPCR method with species-specific primers and probe sets labeled with 5′-carboxyfluorescein (FAM) and 3′-fluorescent quencher (HEX_3) fluorescent dyes. The analysis was performed using Analytik-Jena patented commercial test kits, which included pure reference DNA from cattle, sheep, and chicken species.

During the analysis process, TaqMan qPCR graphs obtained using primers specific to cattle and sheep species, along with a FAM probe specific to mammals, yielded positive results for cattle and sheep in all analyzed meat products (Table 3/A3, B3, C3, D3, E3, F3). In contrast, no positive findings were detected in the TaqMan qPCR analysis using the FAM probe specific to chicken for the three examined meat products (Table 4/A3, B3, C3, D3, E3, F3). However, as expected, the chicken-specific FAM Positive Control probe, which serves as a commercial kit standard, produced a positive signal (Table 4/C4, D4). Based on these data, it was determined that the meat products in question originated from cattle and sheep.

To ensure the accuracy and reliability of the study results, species-specific primers and probes were used with the TaqMan qPCR method, and simultaneously, Positive Control (PK) analyses were performed with the Analytik-Jena kit standard, yielding positive results (Table 3-4/C4, D4). Additionally, to avoid false-negative amplification, Extraction Negative Control (ENC), Environmental Control (CK), and Negative Test Control (NTC) criteria were applied, and as expected, all analyses yielded negative results (Table 3-4/A4, B4, E4, G3, H3). These data confirm that the analysis results are completely accurate and reliable.



Figure 1: Sausages, a red meat product purchased from three different butchers in Ödemiş (İzmir) district (original)

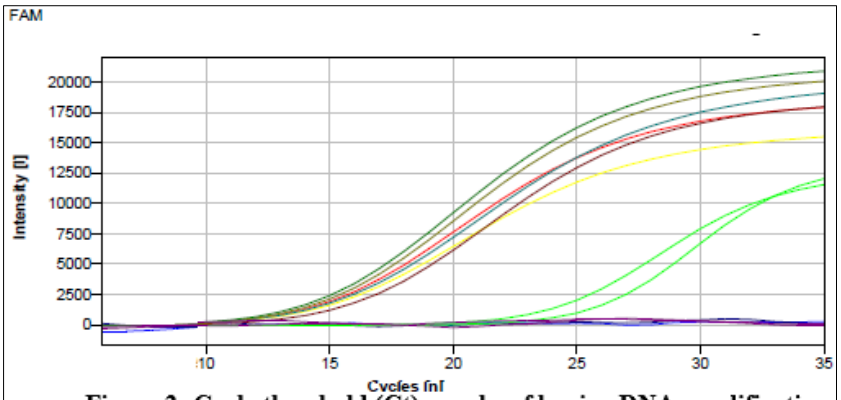


Figure 2: Cycle threshold (Ct) graphs of bovine DNA amplification products (PCR) from research samples using the bovine-specific FAM probe

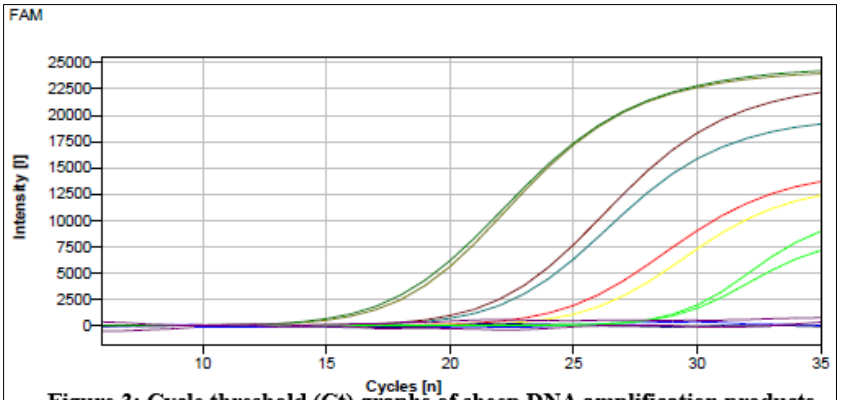


Figure 3: Cycle threshold (Ct) graphs of sheep DNA amplification products (PCR) specific to the sheep FAM probe in research samples

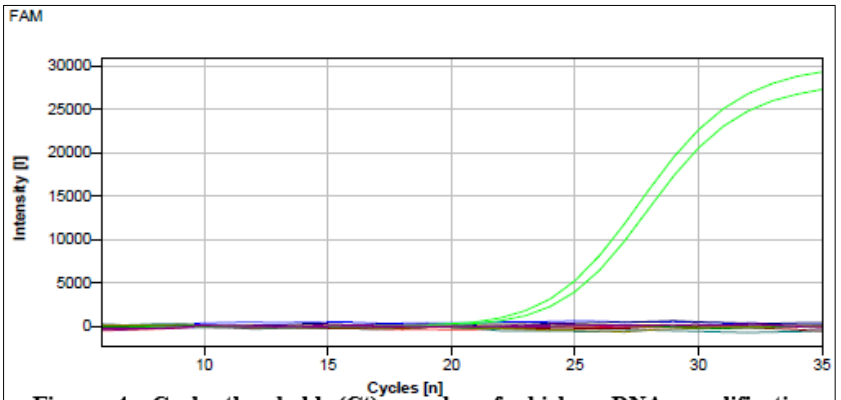


Figure 4: Cycle threshold (Ct) graphs of chicken DNA amplification products for the chicken-specific FAM probe

Table 1: TaqMan qPCR mastermix contents

Reagents	Amount
2xMastermix	10 L
Primer/Probe mix beef IC	3 L
Internal control	1 L
Sample DNA	5 L
PCR Grade water	Complete to 20 L.

Table 2: TaqMan qPCR thermal cycling times and temperatures

Stages	Temperature	Time	Cycle number
Denaturation	95°C	2 minutes	1
Annealing	65°C	10 seconds	35
Extension	72°C	45 seconds	

Table 3: Cycle threshold (Ct) data of cattle, sheep, and chicken DNA amplification products (PCR) from research samples

Prob	Gen Cytb	Meat Product / Sample No	Analysis No	C_T values	Mean C_T values	General C_T values
FAM	Cattle	Sucuk /Odm-1	21950a	13,31	13,20	13,25
			21950b	13,09		
		Sucuk /Odm-2	21951a	13,45	13,96	
			21951b	14,47		
		Sucuk /Odm-3	21952a	13,12	12,96	
			21952b	12,80		
	Sheep	Sucuk /Odm-1	21950a	23,56	24,17	23,50
			21950b	24,78		
		Sucuk /Odm-2	21951a	20,64	20,35	
			21951b	20,07		
		Sucuk /Odm-3	21952a	16,17	15,95	
			21952b	15,73		
	Chicken	Sucuk /Odm-1	21950a	0,0	0,0	0,0
			21950b	0,0		
		Sucuk /Odm-2	21951a	0,0	0,0	
			21951b	0,0		
		Sucuk /Odm-3	21952a	0,0	0,0	
			21952b	0,0		

Table 4: Specificity test results of cattle DNA amplification products (PCR) from research samples with cattle-specific FAM and HEX_3 (ITS) probes

Well		Sample name	Sample type	Dye	Gene	Ct
A1		21950a	U	HEX_3	IPC	No Ct
A1		21950a	U	FAM	SIĞIR	13,31
A2		ENK	U	HEX_3	IPC	20,78
A2		ENK	U	FAM	SIĞIR	No Ct
B1		21950b	U	HEX_3	IPC	No Ct
B1		21950b	U	FAM	SIĞIR	13,69
B2		CK	U	HEX_3	IPC	20,45
B2		CK	U	FAM	SIĞIR	No Ct
C1		21951a	U	HEX_3	IPC	No Ct
C1		21951a	U	FAM	SIĞIR	13,45
C2		PK	+	HEX_3	IPC	20,06
C2		PK	+	FAM	SIĞIR	23,29
D1		21951b	U	HEX_3	IPC	No Ct
D1		21951b	U	FAM	SIĞIR	14,47
D2		PK	+	HEX_3	IPC	20,15
D2		PK	+	FAM	SIĞIR	25,01
E1		21952a	U	HEX_3	IPC	No Ct
E1		21952a	U	FAM	SIĞIR	13,12
F1		21952b	U	HEX_3	IPC	No Ct
F1		21952b	U	FAM	SIĞIR	12,8
G1		NTC	N	HEX_3	IPC	20,54
G1		NTC	N	FAM	SIĞIR	No Ct
H1		NTC	N	HEX_3	IPC	20,72
H1		NTC	N	FAM	SIĞIR	No Ct

Table 5: Species specificity test results of sheep DNA amplification products (PCR) from research samples using sheep-specific FAM and HEX_3 (ITS) probes

Well		Sample name	Sample type	Dye	Gene	Ct
A5		21950a	U	HEX_3	IPC	21,44
A5		21950a	U	FAM	KOYUN	23,56
A6		ENK	U	HEX_3	IPC	21,04
A6		ENK	U	FAM	KOYUN	No Ct
B5		21950b	U	HEX_3	IPC	21,44
B5		21950b	U	FAM	KOYUN	24,78
B6		CK	U	HEX_3	IPC	21,69
B6		CK	U	FAM	KOYUN	No Ct
C5		21951a	U	HEX_3	IPC	21,4
C5		21951a	U	FAM	KOYUN	20,64
C6		PK	+	HEX_3	IPC	21,25
C6		PK	+	FAM	KOYUN	29,09
D5		21951b	U	HEX_3	IPC	21,24
D5		21951b	U	FAM	KOYUN	20,07
D6		PK	+	HEX_3	IPC	21,35
D6		PK	+	FAM	KOYUN	28,84
E5		21952a	U	HEX_3	IPC	22,01
E5		21952a	U	FAM	KOYUN	16,17
F5		21952b	U	HEX_3	IPC	No Ct
F5		21952b	U	FAM	KOYUN	15,73
G5		NTC	N	HEX_3	IPC	21,13
G5		NTC	N	FAM	KOYUN	No Ct
H5		NTC	N	HEX_3	IPC	20,91
H5		NTC	N	FAM	KOYUN	No Ct

Table 6: Species-specific test results of chicken DNA amplification products (PCR) from research samples using chicken-specific FAM and HEX_3 (ITS) probes

Well		Sample name	Sample type	Dye	Gene	Ct
A3		21950a	U	HEX_3	IPC	21,22
A3		21950a	U	FAM	TAVUK	No Ct
A4		ENK	U	HEX_3	IPC	20,65
A4		ENK	U	FAM	TAVUK	No Ct
B3		21950b	U	HEX_3	IPC	21,28
B3		21950b	U	FAM	TAVUK	No Ct
B4		CK	U	HEX_3	IPC	21,01
B4		CK	U	FAM	TAVUK	No Ct
C3		21951a	U	HEX_3	IPC	21,1
C3		21951a	U	FAM	TAVUK	No Ct
C4		PK	+	HEX_3	IPC	20,5
C4		PK	+	FAM	TAVUK	22,6
D3		21951b	U	HEX_3	IPC	20,7
D3		21951b	U	FAM	TAVUK	No Ct
D4		PK	+	HEX_3	IPC	20,62
D4		PK	+	FAM	TAVUK	22,04
E3		21952a	U	HEX_3	IPC	21,33
E3		21952a	U	FAM	TAVUK	No Ct
F3		21952b	U	HEX_3	IPC	21,42
F3		21952b	U	FAM	TAVUK	No Ct
G3		NTC	N	HEX_3	IPC	21,23
G3		NTC	N	FAM	TAVUK	No Ct
H3		NTC	N	HEX_3	IPC	21,09
H3		NTC	N	FAM	TAVUK	No Ct

DISCUSSION

Nowadays, cases of meat adulteration include foods such as sausages, burger patties, and meat-filled pastries. These are processed meat products and are commercially available on store shelves. Since their original physical appearance has been significantly altered, it is nearly impossible to determine their origin without monitoring and examining them through laboratory tests. Various processing techniques can change many sensory attributes of meat products, including volume, appearance, color, texture, smell, and taste, making it difficult to distinguish them physically (Gómez *et al*., 2020). In this study, sausages obtained from butcher shops selling commercial meat products in the Ödemiş district (İzmir) were examined to determine their origins using DNA-based identification.

With the increasing demand for animal protein and consumers becoming more aware of food composition, accurately identifying the source of food has become essential. In recent years, DNA-based techniques have replaced other traditional methods for species identification in meat products. The use of DNA-based methods has enhanced detection specificity and sensitivity. One of the fast, cost-effective, and reliable analytical methods for identifying the origin of meat products is the TaqMan qPCR technique (Adenuga and Montowska, 2023). In this study, the TaqMan qPCR technique was applied to determine the origin of sucuk-type meat products.

Real-time qPCR technique is a highly powerful method for species identification in terms of both specificity and sensitivity. The specificity of the method is related to the specificity of the well-designed primers and probes. In previous species identification studies using the PCR technique in meat and meat products, mitochondrial DNA is generally selected as the target region due to its species-specific loci. In mitochondrial DNA, genes such as cytochrome b, D-loop, 12S rRNA, and 16S rRNA are commonly used for species identification (Rodriguez *et al*., 2005). In the present study, species-specific primers designed for the cytochrome b gene locus were used to identify the origins of commercial sausage products obtained from butcher shops in Ödemiş (İzmir), and successful results were obtained. As a result of the study, it was determined that the analyzed sausages originated from beef and mutton, and no presence of poultry meat was detected.

Using the TaqMan qPCR method, nucleic acid loads in research samples can be detected at low levels. Rodriguez *et al*., (2005) determined species type using the TaqMan probe system targeting mitochondrial 12S rRNA with a nucleic acid quantity as low as 0.1% (0.01 ng). Chisholm *et al*., (2005) detected horse and donkey species in commercial products with nucleic acid quantities of 1 pg and 25 pg, respectively. Mendoza-Romero *et al*., (2004) identified bovine DNA at an extremely low level of 10 fg (=9.78×10^5 base pairs) using a real-time PCR technique targeting 88 bp fragments of a nuclear element. In the present study, species origin was determined using the TaqMan q-PCR method with species-specific primers and probes (Analytik-Jena) contained in a commercial kit, detecting nucleic acid amounts as low as 0.01 ng.

The cycle threshold (Ct) is the number of thermal cycles at which the fluorescence signal surpasses the background signal. The Ct value is a semi-quantitative parameter that helps distinguish between high and low target nucleic acid loads. It is inversely proportional to the amount of target nucleic acid present in the sample (Rao *et al.,* 2020). The lower the Ct value, the higher the amount of target genetic material in the sample. Generally, results with a Ct value of ≤33 are considered positive (Demir-Önder *et al*., 2022).

Within the scope of this study, the average Ct values specific to cattle and sheep species in the examined meat products were calculated as 13.25 and 23.50, respectively. Since these Ct values are below the threshold value of <33, the results are considered reliable. Additionally, the Ct value for chicken was found to be negative in the examined meat products, indicating that the analyzed meat products originated from red meat sources.

Magleby *et al*., (2021) classified nucleic acid load values in analyzed products into three groups: Ct <25 as "high load," Ct = 25-30 as "medium load," and Ct >30 as "low load." Accordingly, the cattle Ct value in commercially available sausage products purchased from butcher shops in Ödemiş (İzmir) was categorized as high load, while the sheep Ct value was lower than that of cattle, indicating a lower load characteristic. These data suggest that cattle origin is more commonly used in the meat products analyzed. Therefore, the Ct <25 “high nucleic acid load” values obtained in the present study strongly support that the analyzed meat products are primarily of cattle origin, with some also containing sheep.

CONCLUSION

As a result, in this study, the bovine and ovine origins of the commercial meat product sucuk sold in butcher shops in Ödemiş (İzmir, Türkiye) were confirmed as stated on the labels. Furthermore, with the help of standard analytical kits, the origin of each species composing the meat products could be easily distinguished from other species, even at a low concentration of 0.01 ng. Additionally, in the context of public health protection, it is recommended that local authorities conduct broader-scale research and inspections to improve the reliability of meat-producing enterprises.

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Author Contributions

This study is based on the Master's thesis of Mehmet ÇAYLALI. The author was responsible for data collection, analysis, and drafting the manuscript. All authors reviewed, edited, and approved the final version.

Conflicts of Interest: The Authors declare that there are no conflicts of interest related to this study.

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