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Bacteriological and Genetic Assessment of Game Meat from Japanese Wild Boars

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Bacterial tests were used to assess bacterial contamination of game meat from Japanese wild boars. The bacterial contamination of wild boar meat was less than that of domestic pork, as determined by aerobic plate counts (APC) and coliform counts. None of the meat examined in this study was contaminated by *Salmonella* or *E. coli* O-157. To detect adulteration by domestic pig meat or European wild boar meat, 46 samples of game meat sold as Japanese wild boar were examined genetically. A total of 17 samples showed genetic haplotypes of European and Asian domestic pigs in the D-loop of mitochondrial DNA (mtDNA), and 16 samples showed nuclear glucosephosphate isomerase-processed pseudogene (*GPIP*) genotypes of European domestic pigs. The European *GPIP* genotypes of these samples were confirmed by PCR−RFLP analysis. These results indicate that some game meat sold as Japanese wild boar is adulterated by cross-breeding between pigs and wild boars or by contamination with meat from domestic pigs or European wild boars.

KEYWORDS: Wild boar; pork; mitochondrial DNA; glucosephosphate isomerase-processed pseudogene (GPIP); PCR−RFLP

INTRODUCTION

Wild boars (*Sus scrofa*) are found throughout the world. Two subspecies of wild boar inhabit Japan: the Japanese wild boar (*S. s. leucomystax*), found on 3 of Japan's 4 main islands (Honshu, Shikoku, and Kyushu); and the Ryukyu wild boar (*S. s. riukiuanus*), which lives only in the Ryukyu Islands (*1*). Japanese wild boars are hunted from November to February in Japan. For centuries, the meat was eaten only by hunters and their families. Today, wild boar meat is considered a delicacy, and in the recent past, it was sold at meat shops for a limited winter period. However, wild boar meat is now sold all year round and can be easily obtained by mail order or via the Internet.

The meat of wild mammals and birds obtained by hunting is known as "game meat". The meat of deer and wild boar is particularly popular in Japan. Nowadays, retailers often stock frozen meat from wild boars that were killed during the hunting season. Wild boar breeding farms have also been established in many prefectures in Japan. Consequently, wild boar meat can now be purchased throughout the year. However, there are no regulations regarding the handling of wild game meat. Domestic animals (Japanese definition: cattle, pig, horse, sheep, goat, and poultry) are required to be slaughtered in an abattoir, and the meat must be inspected by qualified inspectors. In contrast, wild game animals are often killed in the field, and the meat is sold without having been inspected. Thus, there are no mechanisms for preventing or detecting contamination of game meat by bacteria. In 1995, there were cases of O-157 infection from venison in Oregon, USA (*2*), and similar O-157 infections from venison occurred in Japan in 1997. Unless preventive measures are taken, many more bacterial infections from game meat are likely to occur in Japan.

Polymerase chain reaction (PCR) techniques have been used for detection of specific species in foods. Quinteiro et al. (*3*) identified fish species in heat-treated canned tuna, using PCR and restriction fragment length polymorphism (RFLP) analysis of the mitochondrial cytochrome *b* gene (*cytb*). Similarly, PCR−RFLP analysis of 12S and 16S ribosomal RNA (rRNA) has been used for intraspecific distinction of snails (*4*). To detect pork in meat products, Montiel-Sosa et al. (*5*) developed a method involving PCR amplification of the D-loop region of mitochondrial DNA (mtDNA) with highly species-specific primers. To distinguish Asian and European pig breeds, the allelic polymorphism of nuclear glucosephosphate isomerase-processed pseudogene (*GPIP*) appears to be a useful marker (*6*).

There is the possibility that products adulterated with cheaper meat are sold as Japanese wild boar meat. Typical levels of bacterial contamination of wild boar meat are not known. In this study, we used bacterial counts to examine boar meat for contamination and used molecular genetic analyses to assess the genetic lineage of meat sold as Japanese wild boar (and thus detect mislabeling). The bacterial tests comprised counting of aerobic bacteria and coliforms and assays for *Salmonella* and

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Table 1. Source and Genetic Type of the 46 Wild Boar Meat Samples

game meat store	prefecture[a]	source	mtDNA haplotype[b,c]	*GPIP* genotype[c]	no. of samples
A	Miyazaki	farmed	J10	*3a/*3a	2
			J10	*3/*3	1
			J15	*3/*3	3
			M27	*1/*1	1
B	Ooita	hunted	J10	*3/*3	6
			J15	*3/*3	1
C	Shimane	farmed	J10	*3/*3	8
D	Hyogo	hunted	J10	*1/*1	2
			J10	*3a/*3a	2
			J15	*1/*1	2
			J15	*3a/*3a	1
			J1	*1/*1	1
E	Shizuoka	farmed (imported)	**M43**	***3/*4**	4
			M30	***4/*4**	4
F	Miyagi	farmed	**M44**	***4a/*4a**	8
Total 6					46

[a] Prefecture indicates the location of game meat stores. [b] mtDNA haplotype previously defined by Ishiguro et al. (9). The designation "J" indicates Japanese wild boar lineage. The designation of "M" refers to wild boar and domestic pigs of non-Japanese lineage. [c] The bold letters indicate domestic pig lineages except Japanese wild boar.

E. coli O-157. Tests of genetic lineage comprised examination of mtDNA D-loop haplotypes and *GPIP* genotypes.

MATERIALS AND METHODS

Samples. Wild boar meat was purchased from 6 stores (designated as stores A−F) in Japan: in Miyazaki, Ooita, Shimane, Hyogo, Shizuoka, and Miyagi prefectures (**Table 1**). We ordered at least 2 separate packages of meat from each store, via the Internet or from shopping catalogues; the meat was delivered in frozen packages. From each package, 3 or 4 samples (ca. 30 g each) were taken, for a total of 46 samples. A total of 20 pieces of meat from domestic pigs were purchased from 4 butcher shops, and one sample (ca. 30 g) was taken from each piece.

Bacteriological Analysis. Approximately 20 g of meat was mixed with 30 mL of sterilized saline using a stomacher. Serial 10-fold dilutions (10^{-1}−10^{-3}) were prepared, and 0.1 mL of each dilution was plated in duplicate on Perlcore agar plates (Eiken, Tokyo, Japan) for aerobic plate count (APC) and on Perlcore desoxycholate agar plates (Eiken) for coliform count. The APC plates were incubated at 37 °C for 48 h, and the desoxycholate agar plates were incubated at 37 °C for 24 h. Bacteria counts were expressed as colony-forming units (CFU)/g of meat.

Meat (2−3 g) was mixed with 10 mL of Hajna tetrathionate broth (Eiken) and incubated at 37 °C for 24 h. Then, 0.1-mL aliquots of this mixture were inoculated onto desoxycholate hydrogen sulfide lactose (DHL) plates to assay for *Salmonella*. White-colored colonies on these DHL plates were transferred to triple sugar iron agar to confirm identification of *Salmonella*.

Using a stomacher, 20 g of meat was mixed with 50 mL of modified EC broth (Kyokuto, Tokyo, Japan), and this mixture was incubated for 24 h. After incubation, 1 mL of this mixture was combined with 4 mL of saline solution and examined for *E. coli* O-157 using a Path-Stik kit (Celsis, Landgraaf, Netherlands).

DNA Extraction, PCR, and DNA Sequencing. Total cellular DNA was extracted from the meat samples using standard protenase-K phenol−chloroform methods, as described by Watanobe et al. (*1*). A 574-bp fragment of the mtDNA control region and a 507-bp fragment of the glucosephosphate isomerase-processed pseudgene (*GPIP*) (*7*) were obtained by polymerase chain reaction (PCR). The primers mitL112 (5′-GCGCACAAACATACAAATATGCTG-3′) and mitH106 (5′-ACGTGTACGCACGTGTACGC-3′) were used (*8*) to amplify the D-loop mtDNA. The primers GPIP1 [5′-TGCAGTTGAGAAGGACTT-TACTT-3′, developed by Giuffra et al. (*6*)] and GPIP6 [5′-GAAGT-TACAGGGCATCATCTTG-3′, developed by Ishiguro et al. (*9*)] were used to amplify the 507-bp *GPIP* fragment. The PCR began with denaturation for 9 min at 95 °C, followed by 50 cycles of replication (30 s at 95 °C, 30 s at 57 °C, and 1 min at 72 °C) and final extension at 72 °C for 7 min. After PCR, the DNA fragment was purified using a Centricon 100 microconcentrator (Amicon, Beverly, MA) and was sequenced directly using the dideoxy chain termination method with 373S and 310 DNA sequencers and Taq Dyedeoxy and Big Dye terminator cycle sequencing kits (Applied Biosystems, Foster City, CA). The primers mitL11 (5′-CCATGCCGCGTGAAACCA-3′) and mitH12 (5′-ATCGAGATGTCTTATTTAAG-3′) were used to sequence the mtDNA D-loop region (*9*), and the primers GPIP1 and GPIP6 were used to sequence *GPIP*. The results of sequencing were analyzed using GENETYX-MAC software (Software Development Co., Tokyo, Japan) for multiple sequence alignment.

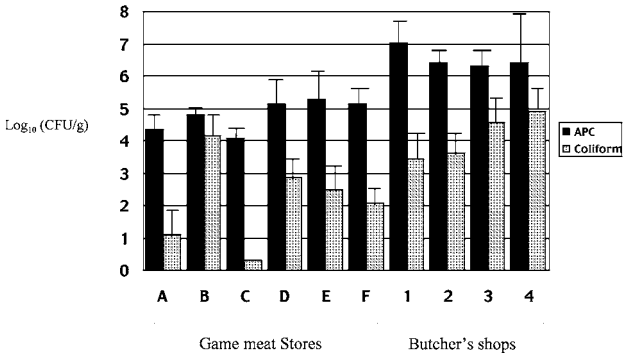

Figure 1. Bacterial contamination of wild boar meat and pork. The letters A–F indicate the stores from which the wild boar meat was purchased, and the numbers 1–4 indicate the butcher shops from which the pork was purchased. Mean APC and coliform count are indicated by the black and hatched columns, respectively.

Phylogenetic Analysis. Sequences of D-loop mtDNA from 303 animals (122 Japanese wild boars, 13 Ryukyu wild boars, 77 East Asian wild boars, 73 European domestic pigs, 3 European wild boars, 3 Northeast Asian domestic pigs, 5 Taiwanese wild boars, and 7 Korean wild boars) were used. All sequences were obtained from the mtDNA database previously published by Okumura et al. (*10*) and Watanobe et al. (*8*). Phylogenetic analysis was performed using the PHYLIP program package (version 3.573; *11*). The mean number of substitutions per nucleotide site was calculated using the 2-parameter method (*12*), after the elimination of nucleotide gaps. From the estimated distance matrixes, dendrograms were created using neighbor-joining (NJ; *13*) methods. The bootstrap method (*14*) was used to determine the confidence interval of each phylogeny from 1000 bootstrap repetitions.

PCR-Restriction Fragment Length Polymorphism (RFLP) Analysis. *GPIP* fragments produced by PCR were subjected to RFLP analysis. The restriction endonucleases *Hha*I and *Bgl*I were used to determine *GPIP* genotypes (as indicated by nucleotide polymorphisms) at nucleotide positions 316 and 233, respectively. Digested samples were electrophoresed in 2% agarose gel, stained with ethidium bromide and photographed under UV light.

RESULTS

Bacteriological Examination of Wild Boar Meat and Pork. The mean APCs (CFU/g) and coliform counts (CFU/g) of the wild boar meat are shown in **Figure 1**. APCs of the 46 samples of wild boar meat ranged from 1.4×10^3 to 1.1×10^6 CFU/g, and APCs of the 20 samples of pork ranged from 4.8×10^3 to 9.7×10^7 CFU/g. The mean APCs of the boar meat were all lower than those of the pork (**Figure 1**).

The mean coliform counts of the wild boar meat varied greatly. The highest coliform count for wild boar meat (for meat from store B) was 5.1×10^4 CFU/g, indicating that this meat was heavily contaminated with coliforms. In contrast, wild boar meat from store C had an estimated coliform count of less than

10 CFU/g (**Figure 1**). The mean coliform counts of wild boar meat from the 5 stores other than store B were lower than those of all the pork examined.

Thus, contrary to our expectations, both APCs and coliform counts of the pork were higher than those of the wild boar meat (excluding the coliform count of wild boar meat from store B).

Neither *Salmonella* nor *E. coli* O-157 was detected in any of the wild boar meat or pork.

Genetic Typing of Wild Boar Meat by PCR−RFLP. The mtDNA haplotypes and GPIP genotypes of 46 wild boar meat samples from 6 stores are shown in **Table 1**. Multiple mtDNA haplotypes and *GPIP* genotypes were detected in 5 of the packages of wild boar meat purchased from stores A, B, and D. This indicates that the game meat within these packages originated from more than one animal.

Nucleotide sequences of mtDNA haplotypes of the wild boar meat were compared with the mtDNA database described above (*8*, *9*) (**Table 2**). Japanese wild boar lineage (abbreviated by J) is distinct from modern wild boar and domestic pigs, including Asian and European lineages (abbreviated by M). Seven haplotypes (J1, J10, J15, M27, M30, M43, and M44) were detected in the 46 wild boar meat samples. To estimate the genetic lineage of these 7 haplotypes, they were analyzed, along with 61 haplotypes from an mtDNA 574-bp database, using the NJ method (*9*), and a phylogenetic tree was constructed (**Figure 2**). The haplotypes J10, J15, and J1 were classified as belonging to Japanese wild boars, and the haplotypes M27 and M30 were classified as belonging to East Asian domestic pigs. The haplotypes M43 and M44, from stores E and F, belong to European domestic pigs that are genetically distinct from Asian lineages. A total of 17 (37%) wild boar meat samples, from stores A, E, and F, contained mtDNA haplotypes that did not belong to Japanese wild boars.

Ishiguro et al. (*9*) reported *GPIP* genotypes for Japanese wild boars, Asian domestic pigs, and European domestic pigs. They found the alleles *GPIP* *1, *GPIP* *3a, and *GPIP* *3 in both Japanese wild boars and Asian domestic pigs (oriental lineage) and found the alleles *GPIP* *4 and *GPIP* *4a in European domestic pigs. The *GPIP* genotypes we found in wild boar meat samples were *GPIP* *1/*1, *3a/*3a, *3/*3, *3/*4, *4a/*4a, and *4/*4 (**Table 1**). We concluded that the genotypes *GPIP* *4a/*4a and *GPIP* *4/*4 had originated from European domestic pigs and that the heterozygous genotype *3/*4 was the result of crossbreeding between oriental and European species. To confirm the presence of European alleles at the nucleotide level, *GPIP* PCR products (507 bp) were digested with *Hha*I or *Bgl*I for PCR−RFLP analysis (**Figure 3**). *Hha*I failed to digest PCR products of genotype *GPIP* *3/*3 (**Figure 3**, lane 1), whereas PCR products of genotype *GPIP* *4/*4 and *GPIP* *4a/*4a were digested by *Hha*I at the guanine at position 316 (**Figure 3**, lanes 3 and 4). *Hha*I digestion of PCR products of heterozygous genotype *GPIP* *3/*4 (4 samples from store E) produced a nondigested 507-bp band and 2 digested bands (**Figure 3**, lane 2). *Bgl*I digested the 507-bp PCR product into 2 DNA fragments (259 bp and 248 bp), cutting it at the cytosine at position 233 (*GPIP* *3/*3, lane 6; *GPIP* *3/*4, lane 7; *GPIP* *4/*4, lane 8). *Bgl*I failed to digest *GPIP* PCR products of allele *GPIP**4a, which has thymine at position 233 (8 samples from store F) (**Figure 3**, lane 9). Thus, the results of PCR−RFLP analyses indicate that the 16 samples from stores E and F contain European *GPIP* genotypes.

After *GPIP* analysis of a sample from store A containing the East Asian mtDNA haplotype M27, we classified it as having the oriental *GPIP* genotype *1/*1. Four samples from store E

Table 2. mtDNA Haplotypes of Wild Boar Meat Examined in This Study[a]

mtDNA haplotype[c]	nucleotide posn[b]																					no. of samples
	15565	15571	15580	15588	15593	15620	15676	15683	15714	15723	15729	15737	15741	15758	15825	15878	15887	15936	15995	16010	16092	16127	16137	
J10	A	–	T	T	G	T	T	G	C	A	G	G	T	T	T	A	T	G	T	G	C	A	G	21
J15	·	–	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	C	·	·	·	·	7
J1	·	–	·	·	·	·	·	·	·	·	·	A	·	·	·	·	·	·	·	·	T	·	A	1
M27	·	–	·	·	·	·	C	·	·	·	·	A	·	·	·	G	·	A	·	·	·	G	·	1
M30	·	–	·	·	·	·	·	·	T	·	·	A	·	·	·	·	C	·	·	·	·	G	·	4
M43	G	C	C	C	A	C	·	A	·	·	A	A	C	·	C	·	C	A	·	A	·	G	·	4
M44	G	C	C	C	A	·	·	·	T	G	A	A	C	C	C	·	C	A	·	A	·	·	·	8

[a] Dots indicate matches with the genotype "J10", and bars indicate deletions. [b] Nucleotide positions are numbered according to the complete pig mtDNA reference (*15*). [c] Haplotypes are quoted from the previous paper (*9*). Haplotypes are the same as **Table 1** and **Figure 2**.

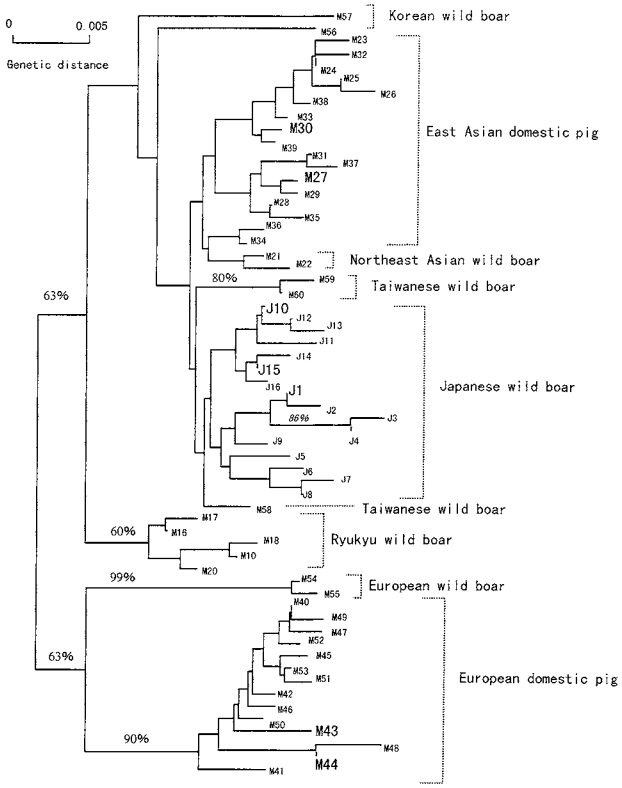

Figure 2. Phylogenetic tree of the partial sequences (574 bp) of the mtDNA control region. The tree was constructed using a neighbor-joining analysis method described elsewhere (*8, 9*). Haplotypes are the same as in **Table 1**. The letter "J" indicates Japanese wild boar lineage, and "M" indicates modern wild boar and domestic pig lineages. The large letter/number combinations indicate the haplotypes of the boar meat examined in this study. Bootstrap resampling was performed 1000 times, and resulting bootstrap probabilities greater than 50% are shown on the corresponding branches.

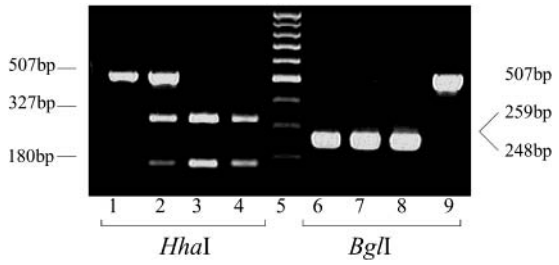

Figure 3. PCR–RFLP of wild boar meat. PCR *GPIP* products were digested with *Hha*I (lanes 1–4) and *Bgl*I (lanes 6–9). The *GPIP* genotypes of the samples were as follows: lanes 1 and 6, *GPIP**3/*3; lanes 2 and 7, *GPIP**3/*4; lanes 3 and 8, *GPIP**4/*4; lanes 4 and 9, *GPIP**4a/*4a. Lane 5 is a 100-bp ladder marker.

containing the European mtDNA haplotype M43 had the crossbred oriental/European *GPIP* genotype *3/*4. Four samples containing the East Asian domestic pig mtDNA haplotype M30 had the European *GPIP* genotype *4/*4. The 8 samples from store F had mtDNA haplotype M44 and *GPIP* genotype *4a/*4a, both of which are of European origin.

DISCUSSION

In Japan, wild game animals are often not slaughtered in an abattoir, and their meat is frequently sold without having been inspected. Given this situation, we expected the level of bacterial contamination of game meat to be greater than that of meat from domestic animals, which are slaughtered in an abattoir. However, we found that meat from Japanese wild boars had a lower level of bacterial contamination than domestic pork, as indicated by APC and coliform count. This finding may be due to differences in the methods of purchasing meat. All the wild boar meat was obtained directly from the producers, by mail order or via the Internet. In addition, all wild boar meat was sent in frozen packages to prevent bacterial growth. The domestic pork was purchased at butcher shops, where the meat is frequently handled during various processes and where the meat may stay for a substantial length of time before being purchased by consumers. The processes that pork undergoes from the abattoir to the butcher shop may be primarily responsible for bacterial contamination of pork.

Furthermore, the game meat examined in this study may have been stored in frozen packages for a long period of time before being shipped. During the freezing process, bacterial populations on game meat may decrease to lower levels than those on raw pork meat. In addition, because sufficient amounts of total DNA were isolated from the frozen game meat, there was no significant difference in PCR results for mtDNA and nuclear *GPIP* genotypes between frozen and raw meat.

Wild boar meat is commercially available throughout the year. However, there is some uncertainty as to whether meat sold as "Japanese wild boar meat" is the genuine article; it is thought that such meat may contain meat from domestic pigs or European wild boars. In this study, genetic analysis was used to determine whether meat sold as Japanese wild boar meat had been adulterated with meat from other sources. We examined the genetic lineage of the wild boars that were processed to game meat using both mtDNA D-loop haplotypes and nuclear *GPIP* genotypes. Montiel-Sasa et al.(*5*) reported that the PCR-amplified mtDNA D-loop region is specific to pigs and useful for detecting both pork meat and fat in mixtures of bovine, ovine, or chicken meat. Furthermore, they also reported that wild boar and pork samples could easily be distinguished by a simple *Ava*II restriction analysis. In this study, mtDNA D-loop haplotype was useful for distinguishing Japanese wild boars from Asian and European domestic pigs but was unable to distinguish Asian domestic lineage from European lineage. Because European and Asian domestic pigs were crossbred in the 18th and 19th centuries to confer beneficial traits to European breeds, modern-day European domestic pigs are sometimes found to possess Asian mtDNA hapotypes. mtDNA is a useful genetic marker to study genetic variation within a species because of a maternal inheritance, but it cannot be used to evaluate paternal influences. To estimate the paternal genetic influence in the hybrid pigs, we examined the nuclear *GPIP* genotypes of wild boar and pork meat.

Multiple genotypes were found within individual samples from 3 stores. This indicates that each of these samples was a mixture of meat from different animals. Out of 46 samples from 6 stores, 17 were classified as having either mtDNA haplotypes or *GPIP* genotypes belonging to lineages other than Japanese wild boars. One sample from store A had East Asian domestic pig mtDNA haplotypes, and all 8 samples from store F had European mtDNA haplotypes and *GPIP* genotypes. The samples from store E had a complicated mix of types: 4 samples had European domestic mtDNA haplotypes and oriental/European crossbred *GPIP* genotypes; 4 other samples had Asian domestic mtDNA haplotypes and European *GPIP* genotypes. The results of this study indicate that Japanese wild boar from farmed sources has been adulterated with European domestic pig or imported meats, while game meat from hunted sources is all Japanese wild boar (**Table 1**).

The main domestic pig species farmed in Japan are Landrace, Large White, and crossbreeds of these 2 species. These breeds were developed in Europe from European wild boars, and they have European domestic genetic types (*6*). The "Inobuta" breed is a cross between Japanese wild boars and female European domestic pigs; it has a European *GPIP* genotype and a European mtDNA haplotype. Therefore, in Japan, it is likely that meat with a European mtDNA haplotype and a European *GPIP* genotype is from "Inobuta" pigs or was imported directly from Europe.

Kodera and Kanzaki (*16*) reported that crossbred wild boars had escaped from farms and given rise to feral Japanese wild boar populations in several areas. The animals examined in the present study may be related to such feral wild boars. The genetic background of wild boar meat can be determined by analyzing mtDNA and *GPIP*. The recent increase in consumption of game meat increases the importance of assessing the quality of such meat, including examination for microbiological contamination. Particularly, crossbreeding between the different lineages or sources of game animals will likely continue to develop on farms soon. To maintain the quality of game meat, rapid and reliable detection methods, such as amplification of mtDNA and nuclear DNA, are needed to expose mixtures with other meats and for species identification.

ABBREVIATIONS USED

PCR, polymerase chain reaction; RFLP, restriction fragment length polymorphism; *GPIP*, glucosephosphate isomerase-processed pseudogene.

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LITERATURE CITED

(1) Watanobe, T.; Okumura, N.; Ishiguro, N.; Nakono, M.; Matsui, A.; Sahara, M.; Komatsu, M. Genetic relationship and distribution of the Japanese wild boar (*Sus scrofa leucomystax)* and Ryukyu wild boar (*Sus scrofa Riukiuanus)* analysed by mitochondrial DNA. *Mol. Ecol.* **1999**, *8*, 1509−1512.

(2) Keene, W. E.; Sazie, E.; Kok, J.; Rice, D. H.; Hancock, D. D.; Balan, V. K.; Zhao, T.; Doyle, M. P. An outbreak of *Escherichia coli* O157: H7 infections traced to jerky made from deer meat. *J. Am. Med. Assoc.* **1997**, *277*, 1229−1231.

(3) Quinteiro, J.; Sotelo, C. G.; Rehbein, H.; Pryde, S. E.; Medina, I.; Pérez-Martín, R. I.; Rey-Méndez, M.; Mackie, I. M. Use of mtDNA direct polymerase chain reaction (PCR) sequencing and PCR-restriction fragment length polymorphism methodologies in species identification of canned tuna. *J. Agric. Food Chem.* **1998**, *46*, 1662−1669.

(4) Borgo, R.; Souty-Grosset, C.; Bouchon, D.; Gomot, L. PCR−RFLP analysis of mitochondrial DNA for identification of snail meat species. *J. Food Sci.* **1996**, *61*, 1−5.

(5) Montiel-Sosa, J. F.; Ruiz-Pesini, E.; Montoya, J.; Roncalés, P.; López-Pérez, M. J.; Pérez-Martos, A. Direct and highly species-specific detection of pork meat and fat in meat products by PCR amplification of mitochondrial DNA.*J. Agri. Food Chem.* **2000**, *48*, 2829−2832.

(6) Giuffra, E.; Kijas, J. M. H.; Amarger, V.; Carlborg, Ö.; Jeon, J.-T.; Andersson, L. The origin of domestic pig: independent domestication and subsequent introgression. *Genetics* **2000**, *154*, 1785−1791.

(7) Harbitz, I.; Chowdhary, B. P.; Kran, S.; Davies, W. Characterization of a porcine glucosephosphate isomerase-processed pseudogene at chromosome 1q1.6−1.7. *Mamm. Genome* **1993**, *4*, 589−592.

(8) Watanobe, T.; Ishiguro, N.; Okumura, N.; Nakano, M.; Matsui, A.; Hongo, H.; Ushiro, H. Ancient mitochondrial DNA reveals the origin of *Sus scrofa* from Rebun Island, Japan. *J. Mol. Evol.* **2001**, *52*, 281−289.

(9) Ishiguro, N.; Naya, Y.; Horiuchi, M.; Shinagawa, M. A genetic method to distinguish crossbred "Inobuta" from Japanese wild boars. *Zool. Sci.* **2002**, *19*, 1313−1319.

(10) Okumura, N.; Kurosawa, Y.; Kobayashi, E.; Watanobe, T.; Ishiguro, N.; Yasue, H.; Matsuhashi, T. Genetic relationship amongst the major noncoding regions of mitochondrial DNAs in wild boars and several breeds of domesticated pigs. *Anim. Genet.* **2001**, *32*, 139−147.

(11) Felsenstein, J. *PHYLIP, Executables for powermac,* **1995**, **v**ersion 3.572.; University of Washington: Seattle, WA, 1995.

(12) Kimura, M. A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequence. *J. Mol. Evol.* **1980**, *16*, 111−120.

(13) Saitou, N.; Nei, M. The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol. Biol. Evol.* **1987**, *4*, 406−425.

(14) Felsenstein, J. Confidence limits on phylogenies: An approach using the bootstrap. *Evolution* **1985**, *39*, 783−791.

(15) Ursing, B. M.; Arnason, U. The complete mitochondrial DNA sequence of the pig (*Sus scrofa). J. Mol. Evol.* **1998**, *47*, 302−306.

(16) Kodera, Y.; Kanzaki, N. Present status of wild boar and crossbred pig × wild boar farming and their feral populations in Japan. *Wildl. Conserv. Jpn.* **2001**, *6*, 67−78 (in Japanese with English abstract).

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