



NOTE

Public Health

The sensitivity of commercial kits in detecting the genes of pathogenic bacteria in venison

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ABSTRACT. The expansion of the wild deer population is a major problem for the Japanese farm and forestry industries because their damage to farm products and vegetation results in huge economic loss. To promote game meat consumption, hygiene inspections should be performed to detect main bacterial pathogens before products are shipped. In this study, we aimed to evaluate the ability of commercial test kits to genetically detect EHEC, *Salmonella* and *Listeria monocytogenes* in venison. Our results demonstrated that the kits for three pathogens could be useful for venison as well as other domestic meat products. Our comparative study showed that the LAMP kits were more sensitive than the RT-qPCR kits in the detection of all of these pathogens.

KEY WORDS: EHEC O157, LAMP, *Listeria monocytogenes*, RT-qPCR, venison

Recently, there have been many reports about the damage that wild animals have caused in relation to the farm industry in Japan. According to these reports, the total amount of damage reached over 20 billion yen, which represents a serious problem [10]. To solve these problems, individual prefectural governments are taking measures to reduce the population of these harmful animals by hunting, while, most carcasses are discarded due to the lack of available slaughter facilities. In addition, Japanese abattoir law is only valid for domestic animals (cattle, swine, horse, sheep, and goats) and does not apply to the slaughter of game animals [11]. Thus, game meats have only been regulated according to the relevant food sanitation acts executed by individual local governments. One of the reasons for the low popularity and inefficient production of game meat is that its safety and hygiene are not considered to be secure. Because the feeding environment of wild animals is not hygienically controlled, it is possible that game meats might contain hazardous substances, such as microorganisms, natural poisons, radioactive contamination, and heavy metals. Additionally, numerous cases of food-borne disease and zoonosis due to game meat consumption have already been reported [1–5, 7, 15, 16]. To improve the level of hygiene control, we propose that commercial test kits would use for the detection of contaminated pathogenic bacteria in venison as a hygiene control method. Among the commercial kits that are used for detecting food pathogens (*i.e.*, immunological, genetic, and culture detection methods), genetic detection methods are most widely applied due to their rapidity and simplicity. Such commercial kits have been validated for general foods, including domestic meat and meat products [6, 9, 12, 13, 17]. However, the efficacy of these kits in detecting pathogens in game meats has not yet been evaluated.

In the present study, we evaluated the specificity of two types commercially available kits (loop amplification method [LAMP] and real-time polymerase chain reaction [RT-qPCR] kits) in the genetic detection of EHEC O157, *Salmonella* Enteritidis, and *Listeria monocytogenes*, which are main causative agents of food-borne disease in people who consume venison [7, 8, 16].

The venison used in the present study was obtained from a Japanese deer (*Cervus nippon centralis*) that was hunted in Nagano prefecture, in central Japan. A block of muscle tissue was carved hygienically from the carcass. After ethanol and

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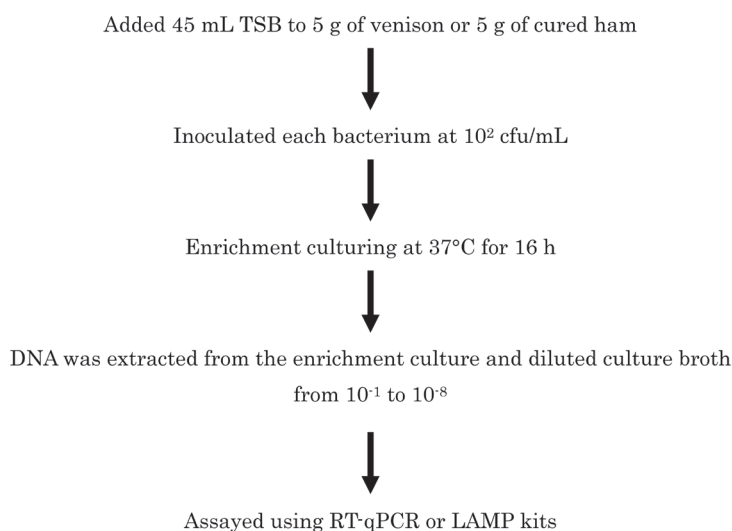


Fig. 1. The protocol used to evaluate the specificity of the genetic detection kits.

Table 1. The specificity of commercial two genetic methods for three pathogens in venison

Method	Bacteria	Target gene	Food sample	Decision ^{a)}	Dilution ^{b)}							
					10 ⁻¹	10 ⁻²	10 ⁻³	10 ⁻⁴	10 ⁻⁵	10 ⁻⁶	10 ⁻⁷	10 ⁻⁸
LAMP	EHEC O157	VT genes	Cured ham	+	+	+	+	+	+	+	+	-
			Vension	+	+	+	+	+	+	+	-	
	<i>L. monocytogenes</i>	iap gene	Cured ham	+	+	+	+	+	+	-	-	
			Vension	+	+	+	+	+	+	-	-	
	<i>S. Enteritidis</i>	inv A gene	Cured ham	+	+	+	+	+	+	+	+	-
			Vension	+	+	+	+	+	-	-	-	
RT-qPCR	EHEC O157	VT genes	Cured ham	+	+	+	+	+	+	+	+	+
			Vension	+	+	+	+	+	+	+	+	-
	<i>L. monocytogenes</i>	inlA gene	Cured ham	+	+	+	+	+	-	-	-	
			Vension	+	+	+	+	+	-	-	-	
	<i>S. Enteritidis</i>	inv A gene	Cured ham	+	+	+	+	+	-	-	-	
			Vension	+	+	+	+	+	-	-	-	

+: positive, -: negative. a) The assay was carried out by DNA extracted from the enrichment culture broth. b) The enrichment culture broth was diluted by TSB.

heat pasteurization, a piece was removed from each side, the central part of the meat was used for the subsequent experiments. Regarding the pathogens, EHEC O157 strain (NIHS 0106 isolated from patient), *Listeria monocytogenes* strain (ALM 14004 isolated from chicken) and *Salmonella* Enteritidis (IID 604) were used.

First, to examine the specificity of these kits for venison, we compared the results obtained from venison and cured ham (one of the meats for which these kits are applicable). The protocol of this experiment is shown in Fig. 1. Venison and cured ham (5 g portions) were placed in Trypticase soy broth (TSB) and inoculated with 10² cfu/ml of each bacteria and incubated for 16 hr at 37°C. DNA was then extracted from the culture broth directly using QIAquick PCR Purification Kit (Qiagen, Hilden, Germany). All three bacteria were detected from the inoculated venison and cured ham using LAMP and RT-qPCR kits according to the manufacturer's instructions. The Loopamp *Escherichia coli* O157 Detection Kit (VT genes, Eiken Chemicals Co., Ltd., Tokyo, Japan) and Cycleave PCR EHEC (VT genes, O157/O26) Typing Kit (TAKARA Bio Co., Kusatsu, Japan) were used to detect EHEC O157. The Loopamp *Salmonella* Detection Kit (inv A gene, Eiken Chemicals Co., Ltd.) and Cycleave PCR *Salmonella* Detection Kit (inv gene, TAKARA Bio Co.) were used to detect *S. Enteritidis*. The Loopamp *Listeria monocytogenes* Kit (iap gene, Eiken Chemicals Co., Ltd.) and Cycleave PCR *Listeria monocytogenes* Detection Kit (inlA gene, TAKARA Bio Co.) were used for the detection of *L. monocytogenes*. The decisions of these kits were positive in all three bacteria after enrichment culture as well as cured ham and when these bacteria were not present in venison, the results of these kits were negative (data not shown), suggesting that these kits have the specificity for detection of these bacteria in venison (Table 1). However since the results were obtained using enrichment culture broth alone, the differences in the sensitivity for each bacterium in cured ham and venison were unclear. Thus, each broth obtained from the enrichment culture was diluted to examine the detection limit. DNA was extracted from each diluted sample using QIAquick PCR Purification Kit and assayed using LAMP and RT-qPCR kits. The LAMP kits detected EHEC O157 and *L. monocytogenes* were detected in both cured ham and venison at 10⁻⁷ and 10⁻⁶ dilution, respectively

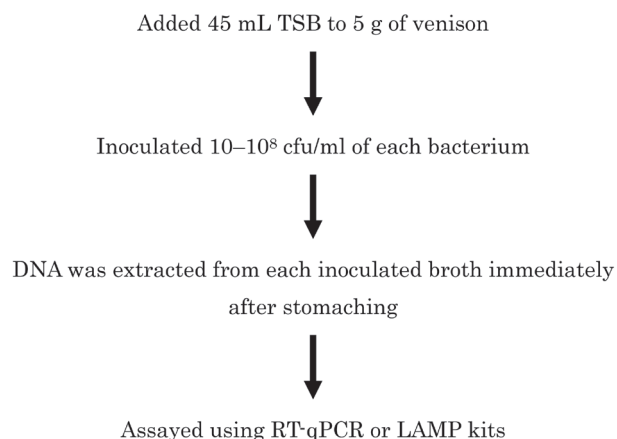


Fig. 2. The protocol of the sensitivity test of the genetic detection kits without enrichment culture process.

Table 2. The sensitivity of commercial two genetic methods for three pathogens in venison without enrichment culture process

Method	Bacteria	Detection limit (cfu/ml) ^{a)}
LAMP	EHEC O157	10 ³
	<i>L. monocytogenes</i>	10 ³
	<i>S. Enteritidis</i>	10 ³
RT-qPCR	EHEC O157	10 ⁷
	<i>L. monocytogenes</i>	>10 ⁷
	<i>S. Enteritidis</i>	>10 ⁷

a) The bacteria were inoculated to TSB containing 10% venison.

(Table 1). *S. Enteritidis* was detected at 10⁻⁷ and 10⁻⁵ dilution in cured ham and venison, respectively. In contrast, in venison, the RT-qPCR kits detected EHEC O157 at 10⁻⁷, while they detected *L. monocytogenes* and *S. Enteritidis* at 10⁻⁵ dilution. In cured ham, EHEC O157 was detected at 10⁻⁸ dilution, while *L. monocytogenes* and *S. Enteritidis* were detected at 10⁻⁵ and 10⁻⁴ dilution, respectively (Table 1). These results suggested that there were no major differences in the sensitivity of the kits for detecting the three pathogens (with the exception of the detection of *S. Enteritidis* using a LAMP kit) in venison and cured ham. There are two possible reasons for the difference observed in the detection of *S. Enteritidis*. Firstly, venison may contain unique constituents that differ from domestic animal meat and which disturb the amplification of DNA. Secondly, the *S. Enteritidis* that contaminate venison show genetic variance and the variance may influence the sensitivity because the commercial kits detect well-known virulence genes in the target pathogenic bacteria and the prominent genes are validated according to the pathogens in domestic meat.

Next, the sensitivity of these methods in the detection of these bacteria without enrichment was examined in venison and beef, pork, and chicken fresh meat if necessary. These fresh meats were purchased from a retail shop in Sagami-hara, Kanagawa, Japan.

Briefly the venison and other fresh meats were cut into 5 g portions and 45 ml of Trypticase-soy broth (TSB) was added and each bacterial culture broth at levels ranging from 10⁸ cfu/ml to 10 cfu/ml in a filtered Stomacher bag and was then stomached for one minute. And then DNA was extracted immediately using QIAquick PCR Purification Kit (Fig. 2). The LAMP kit detected all three bacteria; 10³ cfu/ml was the limit of detection (Table 2). In contrast, the limit of detection of the RT-qPCR for EHEC O157 was 10⁷ cfu/ml while that for *S. Enteritidis* and *L. monocytogenes* was 10⁸ cfu/ml (Table 2). These results indicated that the LAMP kits were more sensitive than the RT-qPCR kits in the detection of these pathogens. In the case that venison was contaminated with these bacteria at >10³ cfu/ml, it would be possible to detect the pathogens with a commercial LAMP kit without an enrichment process. While RT-qPCR showed less sensitivity for all these bacteria in a direct assay. In order to examine the reasons why the sensitivity of the RT-qPCR for these pathogens in venison was reduced in comparison to the LAMP kit, the sensitivity for these pathogens in other meats (beef, pork and chicken) was investigated. Regarding the EHEC O157, the sensitivity in raw ground beef was reported to be 10³ cfu/ml, [14], suggesting that the sensitivity in venison would be inferior to that in beef. For *Listeria*, the sensitivity in beef, pork and chicken was 10⁶, 10⁷ and 10⁷, respectively (data not shown), For *Salmonella*, the sensitivity in beef and pork was the same as that in venison, while that in chicken meat was 10⁵. Based on these results, the lipid content of meat might affect the sensitivity of the RT-qPCR for *Salmonella*.

In conclusion, commercially available LAMP and RT-qPCR kits can be used for the genetic detection of EHEC O157, *S. Enteritidis* and *L. monocytogenes* in venison after enrichment culturing for 16 hr. Furthermore, the LAMP kits showed superior sensitivity to the RT-qPCR kits in the detection of these pathogenic bacteria. The use of commercial kits for hygiene control would contribute to expanding the market for venison.

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