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Detection of *Sarcocystis* spp. and Shiga toxin-producing *Escherichia coli* in Japanese sika deer meat using a loop-mediated isothermal amplification-lateral flow strip

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ABSTRACT. Game meat potentially harbors a number of parasitic and bacterial pathogens that cause foodborne disease. It is thus important to monitor the prevalence of such pathogens in game meats before retail and consumption to ensure consumer safety. In particular, *Sarcocystis* spp. and Shiga toxin-producing *Escherichia coli* (STEC) have been reported to be causative agents of food poisoning associated with deer meat consumption. To examine the prevalence of these microbiological agents on-site at a slaughterhouse, the rapid, simple and sensitive detection method known as the "DNA strip" has been developed, a novel tool combining loop-mediated isothermal amplification and a lateral flow strip. This assay has achieved higher sensitivity and faster than conventional PCR and is suitable for on-site inspection.

KEY WORDS: deer meat, DNA strip, loop-mediated isothermal amplification, *Sarcocystis* spp., STEC

Due to increasing numbers of sika deer (*Cervus nippon*) in Japan, the government recommends hunting these animals and consuming their meats as game meat in local restaurants and retail meat shops [12]. However, since Japanese abattoir law does not apply to the slaughter of game animals, the hunters must dissect and inspect the game by themselves. To guarantee the meat's sanitation, a easy and quick assay that can be used during the slaughter of game animals to determine the presence of foodborne agents is needed. Many rapidly assay methods have been developed for foodborne bacteria, viruses and parasites, including immunochemical assays, conventional polymerase chain reaction (PCR), real time PCR and the loop-mediated isothermal amplification (LAMP) assay [18, 21, 28]. However, few of these methods have been validated using deer meat.

Outbreaks of food poisoning have been caused by parasites, bacteria and viruses associated with the consumption of deer meat in Japan [1, 15, 24]. We recently reported that *Sarcocystis* spp. was one of such causative agents of food poisoning [14]. Kabeya *et al.* [13] also reported that Shiga toxin-producing *Escherichia coli* (STEC) detected in the feces of sika deer possessed potential human pathogenicity. Due to the high contamination frequency in meat or feces in Japan, *Sarcocystis* spp. and STEC [9, 13] were chosen as the subjects in this study.

Given this background, we developed an easy, rapid and sensitive assay for the detection of *Sarcocystis* spp. and STEC in deer meat. The assay is called the "DNA strip" [23] and may be useful for on-site inspection in slaughterhouses.

The "DNA strip" enables the detection of the amplification products by LAMP on a lateral flow DNA strip and has been validated by Takasaki *et al.* [23]. Specific primers for the detection of *Sarcocystis* spp. and STEC were designed by attaching unique oligonucleotides as a tag modification at the 5-terminal of the forward inner primer, while the 5′-terminal of the backward inner primer was biotin-modified. In this way, successfully amplified target DNA will have unique oligonucleotide tags attached and bind to immobilized complementary tags on the DNA strip. The biotin will bond to streptavidin provided in the DNA strip

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Table 1. The sequences and modification of the LAMP and PCR primers used in this study

Method	Assay	Primar name	Sequence	Ref.
DNA strip	18S rRNA of *Sarcocystis* spp. (*SarcoR*)	F3	TGAAAACCTTGGCCGATAGG	This study
		B3	CCTTGTTACGACTTCTCCTTCCTC	
		FIP	[tag]-GTAATCGGCGCAAGCTGCTGACGGTAATCTTTTGAGTATGCATCG	
		BIP	[bi]-TGTACACACCGCCCGTCGCTCTTTCCATTCCGCACACGTTG	
		LF	CTACTAGGCATTCCTCGTTGAAGAT	
		LB	CTACCGATTGAGTGTTCCGGTGA	
	stx1	F3	GCGATTTATCTGCATCCCCGTATGTCTGGTGACAGTAGCTAT	[4]
		B3	GGAACCTCACTGACGCAGTCCTTCAGCTGTCACAGTAACA	
		FIP	ACTGATCCCTGCAACACG	
		BIP	TGTGGCAAGAGCGATGTT	
		LF	ACAACAGCGGTTACATTGT	
		LB	GATCATCCAGTGTTGTACGAA	
	stx2	F3	GGCGTCATCGTATACACAGGAGCGCTTCAGGCAGATACAG	
		B3	AGACGTGGACCTCACTCTGAAACTCTGACACCATCCTCTC	
		FIP	CAGACAGTGCCTGACGAA	
		BIP	GGCGAATCAGCAATGTGC	
		LF	GCATCCAGAGCAGTTCTG	
		LB	CAGTATAACGGCCACAGTC	
Conventional PCR	18S rRNA of *Sarcocystis* spp. (*SarcoR*)	F	GGATAACCGTGGTAATTCTATG	[18]
		R	TCCTATGTCTGGACCTGGTGAG	
	stx1	mStx1_F	GGATAATTTGTTTGCAGTTGATGTC	[17]
	stx1	mStx1_R	CAAATCCTGTCACATATAAATTATTTCGT	
	stx2	mStx2_F	GGGCAGTTATTTTGCTGTGGA	
	stx2	mStx2_R	GAAAGTATTTGTTGCCGTATTAACG	

buffer, generating blue color enhancement.

First, to confirm the performance of the "DNA strip" for the detection of 18S rRNA of *Sarcocystis* spp. (*SarcoR*) as well as the Shiga toxin genes (*stx1* and *stx2*) of STEC, a LAMP assay was performed using the *Sarcocyctis* spp. and STEC isolates in PBS solution. Cyst of *Sarcocystis* spp. was collected from deer meat obtained from Yamanashi Prefecture, Japan. The STEC O157:H7 strain NIHS0106, which possesses both the *stx1* and *stx2* genes, was used as a positive control. These DNA were extracted using a QIAamp DNA mini kit (QIAGEN, Hilden, Germany) according to the manufacturer's instructions. The DNA concentration was measured by a NanoDrop 1000 spectrophotometer (Thermo Fisher Scientific Inc., Wilmington, DE, U.S.A.), and the extracted DNA was prepared at 10-fold dilution to be amplified with the reaction mixture. The reaction mixture contained of 1.5x Isothermal Master Mix (OptiGene Ltd., West Sussex, U.K.), tagged specific primer for the detection of *Sarcocyctis* spp. and STEC, respectively, and 10 × template DNA. DNA amplification was performed for 60 min at 65°C. The tubes were put on ice for a minimum of 5 min to avoid contamination by completely stopping the reaction and performing evaporation at the end of the amplification. The amplification product (1 μl) was diluted 40-fold with distilled water and then mixed with 20 μl of development solution containing Latex beads (TBA Co., Ltd., Sendai, Japan). The DNA strip was dipped into the solution for 15 min, and the results were determined visually. The specific primer of *SarcoR* in *Sarcocystis* spp. and *stx1* and *stx2* in STEC for the LAMP assay are shown in Table 1.

According to a previous paper, some species of *Sarcocystis* have been parasitic to Japanese sika deer [9], such as *S. wapiti*, *S. sybillensis*, and *S. hofmanni*. However, except for *S. wapiti*, the DNA sequences of these species have never deposited in databases, so the sequences of the predominant *Sarcocystis* species in deer meat obtained from Yamanashi Prefecture, Japan, were determined in order to construct LAMP primers for *SarcoR*.

The 18S rRNA region was PCR-amplified from DNA extracted from deer meat using universal 18S rRNA region primers, as described by Pritt *et al*. [20], that were able to detect the 18S rRNA region of *Sarcocystis* spp. universally (Table 1). We then followed by cloning into pCR™ 2.1-TOPO® in *E. coli* TOP10 obtained from the TOPO® TA Cloning Kit for Sequencing (Thermo Fisher Scientific Inc.). The successful transformants were then subjected to a cycle sequencing reaction using the BigDye terminator in an ABI 3730×l system to determine the target gene sequences. Finally, six sequences were obtained (LC405946-LC405951), and the LAMP primers were designed based on the obtained consensus sequences using a LAMP Designer (OptiGene, Ltd.). We used the oligonucleotide primers for the *stx1* and *stx2* genes reported elsewhere [6]. As shown in Fig. 1, we first evaluated the detection performance of the three target genes by LAMP-DNA strip testing. The results indicated that these genes could be discriminated on the DNA strip based on the attached tag sequences.

Next, to determine the limit of detection (LOD) of this assay for both targets, LODs of conventional PCR for these targets were compared using various concentrations of bradizoids and the STEC strain. Conventional PCR for the rRNA of *Sarcocystis* spp.

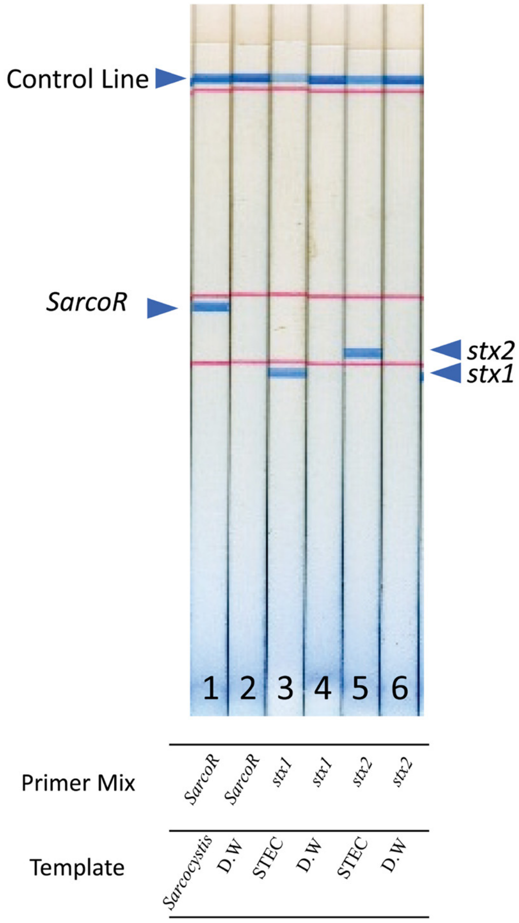

Fig. 1. Performance of the DNA strip for the detection of the *Sarcocystis* spp. 18S rRNA gene (*SarcoR*) and the *stx1* and *stx2* genes. The template DNA was prepared from a cyst of *Sarcocystis* spp. or STEC O157:H7 strain NIHS0106. The primer mix:1,2: *Sarco R*, 3,4: *stx 1*, 5,6: *stx 2* and 1,3 and 4 was with template. The arrow indicates each LAMP product.

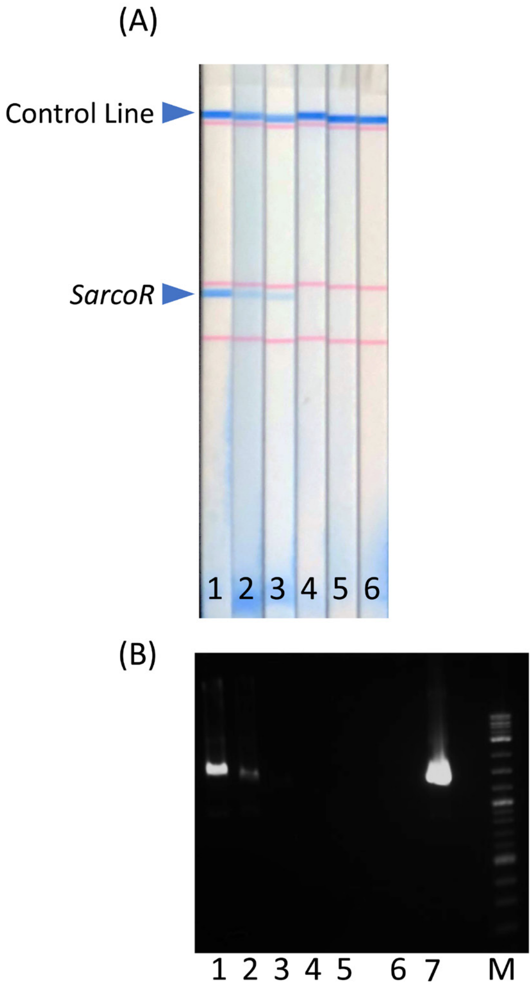

Fig. 2. Sensitivity of (A) the DNA strip and (B) conventional PCR forthe detection of the 18S rRNA gene of *Sarcocystis* spp. 1: 3.6 × 10^5 bradizoids/m*l* in 10% deer meat extract, 2: 3.6 × 10^4 bradizoids/m*l* in 10% deer meat extract, 3: 3.6 × 10^3 bradizoids/m*l* in 10% deer meat extract, 4: 3.6 × 10^2 bradizoids/m*l* in 10% deer meat extract, 5: 3.6 × 10^1 bradizoids/m*l* in 10% deer meat extract, 6: negative sample (10% deer meat extract only), 7: positive sample (Sarcocyst) in PBS. M: molecular marker, *SarcoR*: *Sarcocystis* spp.18S rRNA gene.

and for the *stx1* and *stx2* genes was performed according to the method of Pritt *et al.* [20] and Nielsen *et al*. [19], respectively. The bradizoids were prepared from *Sarcocystis* spp. according to the previous paper [9]. The *Sarcocystis* spp. (3.6 × 10^5 bradizoids/m*l*) or STEC strain (3.4 × 10^8 cfu/m*l*) was added to PBS containing 10% deer meat extract that had been confirmed to be free from *Sarcocystis* spp. as well as STEC and the solutions were diluted serially. The DNA in these dilutions was extracted using a QIAamp DNA mini kit (QIAGEN). In STEC, all serial dilutions of extracted DNA were used for this assay and conventional PCR. In *Sarcocystis* spp., dilutions from 3.6 × 10^5 to 3.6 × 10^1 bradizoids/m*l* were used for both assays. As a negative control, 10% deer meat extract alone in PBS was used. As a positive control for conventional PCR, 3.4 × 10^8 cfu/m*l* in PBS for STEC or a cyst of *Sarcocystis* spp. with 3.6 × 10^6 bradizoids/m*l* (data not shown) in PBS was used.

As shown in Fig. 2, the visual LOD of bradizoids in this assay was determined to be 3.6 × 10^3 bradizoids/m*l* (Fig. 2A), which was 100 times more efficient than that of conventional PCR (Fig. 2B). The negative control showed no visual signals. The difference in the sensitivity seemed to be due to the amplification efficiency and detection procedure.

The LODs of the *stx1* and *stx2* genes with the DNA strip were 3.4 × 10^4 and 3.4 × 10^3 cfu/m*l* of STEC, respectively (Fig. 3A). In contrast, the LODs of these genes with conventional PCR were 3.4 × 10^6 cfu/m*l* of STEC for both targets (Fig. 3B). The results revealed that the DNA strip showed 100- and 1000-fold greater sensitivity for the *stx1* and *stx2* genes, respectively, than conventional PCR. Our previous study revealed that the LAMP assay showed increased sensitivity for the detection of the *stx1* and *stx2* genes from STEC O157 compared with real-time PCR [26]. The difference in sensitivity is likely related to the high

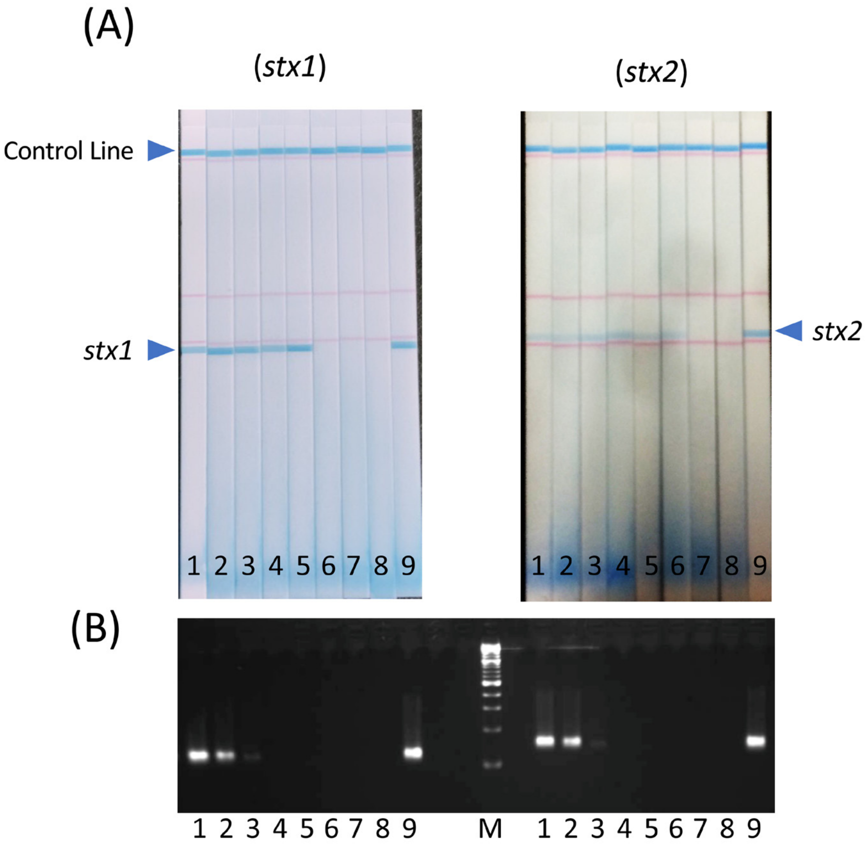

Fig. 3. Sensitivity of (A) the DNA strip and (B) conventional PCR for the detection of *stx1* and *stx2* genes. 1: 3.4×10^8 CFU/m*l* in 10% deer meat extract, 2: 3.4×10^7 CFU/m*l* in 10% deer meat extract, 3: 3.4×10^6 CFU/m*l* in 10% deer meat extract, 4: 3.4×10^5 CFU/m*l* in 10% deer meat extract, 5: 3.4×10^4 CFU/m*l* in 10% deer meat extract, 6: 3.4×10^3 CFU/m*l* in 10% deer meat extract, 7: 3.4×10^2 CFU/m*l* in 10% deer meat extract, 8: negative sample (10% deer meat extract only), 9: positive sample (3.4×10^8/m*l* in PBS), M: molecular marker.

Table 2. The specificity of DNA strip for detection of *Sarcocystis* spp. and STEC in Japanese sika deer

Subject of DNA strip	Strain	Species	Cross reaction
STEC	PE7	EAEC[a)]	Negative
	HP1001	EPEC[b)]	Negative
	WHO1	ETEC[c)]	Negative
	NIHS_00214	STEC O157: H7 *stx* (−)	Negative
	NIHS_00069	*Salmonella* Enteritidis	Negative
	ATCC8739	Commensal *E. coli*	Negative
	ATCC43864	*Citrobacter freundii*	Negative
	ATCC10145	*Pseudomonas aeruginosa*	Negative
	Lm0132	*Listeria monocytogenes*	Negative
Sarcocystis spp.	AFSS-0002	*Sarcocystis fayeri*	Positive
	RH strain (NIHS)	*Toxoplasma gondii*	Positive
	AV-Tp001	*Theileria parva*	Negative

a) EAEC: enteroaggregative *Escherichia coli*, b) EPEC: enteropathogenic *Escherichia coli*, c) ETEC: enterotoxigenic *Escherichia coli*.

performance of the LAMP procedure in the DNA strip.

The specificity of the DNA strip for each hazard was examined. To validate the specificity for *stx1* and *stx2* genes in STEC, DNA extracts obtained from genera non-Shiga toxin-producing *E. coli* and other pathogenic bacteria were used. To investigate the specificity of *Sarcocystis* spp. in Japanese sika deer, DNA extracts obtained from the closely related genera (*Toxoplasma gondii*, *Theileria parva*) and *Sarcocystis fayeri* were used. As shown in Table 2, none of the bacterial DNA extracts used in this experiment showed cross reactions, suggesting that the DNA strip had high sensitivity for the *stx1* and *stx2* genes in STEC. However, the DNA strip showed cross-reactions for *T. gondii* and *S. fayeri,* but not *T. parva,* demonstrating that our primer reacted with the 18S

Table 3. Comparison of conventional PCR and DNA strip for detection of *Sarcocyctis* spp., *Toxoplasma gondii* and STEC in Japanese sika deer

Sample No.	Sex	*Sarcocystis* spp.		*Toxoplasma gondii*	STEC	
		Conventional PCR	DNA strip	ELISA	Conventional PCR	DNA strip
1	M	-	-	NT	-	-
2	F	-	-	NT	-	-
3	M	-	-	NT	-	-
4	M	-	-	NT	-	-
5	F	-	-	NT	-	-
6	M	-	+	NT	-	-
7	M	-	-	NT	-	-
8	F	+	+	NT	-	-
9	F	+	+	-	-	-
10	F	+	+	-	-	-
11	F	-	-	-	-	-
12	M	-	-	-	-	-
13	F	+	+	-	-	-
14	F	+	+	NT	-	-
15	F	+	+	-	-	-
16	M	+	+	-	-	-
17	F	+	+	-	-	-
18	F	+	+	-	-	-
19	M	+	+	-	-	-
20	F	+	+	-	-	-
21	M	+	+	-	-	-
22	M	+	+	-	-	-
23	M	+	+	-	-	-
24	M	-	+	-	-	-
25	M	-	+	-	-	-
26	F	+	+	-	-	-
27	F	+	+	-	-	-
28	F	+	+	-	-	-
29	F	+	+	-	-	-
30	F	+	+	-	-	-
31	F	-	+	-	-	-
32	F	+	+	-	-	-
33	F	-	-	-	-	-
34	F	+	+	-	-	-
35	F	+	+	-	-	-
36	M	+	+	-	-	-
37	F	+	+	-	-	-
38	F	+	+	-	-	-
39	M	+	+	-	-	-
40	F	-	+	-	-	-
41	unknown	+	+	-	-	-
42	M	+	+	-	-	-
43	F	-	-	-	-	-
44	M	+	+	-	-	-
45	unknown	+	+	-	-	-
46	unknown	+	+	NT	-	-
47	unknown	+	+	NT	-	-
Total number of positive sample		32	37	0	0	0

NT, not tested; +, positive; -, negative.

rRNA of *T. gondii and S. fayeri* because the 18S rRNA of these parasites were similar. And *T. gondii* has been reported to infect in Japanese sika deer [4] and cause food poisoning [16]. The results also suggested that this DNA strip might be applicable to the detection of *Sarcocystis* spp. in other animals, including wild deer and *T. gondii.*

Finally, we assessed the prevalence of *Sarcocystis* spp. and STEC in a total of 47 samples of meat from deer that had been slaughtered and processed in Yamanashi Prefecture, Japan using the DNA strip in combination with the conventional PCR approach. *Toxoplasma gondii* in serum was examined for 36 samples out of 47 samples using commercial ELISA kit (ID Screen®

Toxoplasmosis Indirect Multi-species, ID.Vet, Grabels, France). As shown in Table 3, 32 samples were positive for conventional PCR while 37 samples were positive for the DNA strip, and *T. gondii* was negative in all samples assayed. Therefore it is considered that the positive samples in this study were contaminated with *Sarcocystis* spp. predominantly. Since the negative samples detected by the DNA strip were also negative on conventional PCR, we concluded that the DNA strip was able to detect *Sarcocystis* spp. from deer meat more efficiently than conventional PCR. Parallel experiments revealed that no STEC were detected in either assay. We previously reported that STEC OUT:H25 was isolated from only 1 out of 120 venison samples [2]. This suggests that the prevalence rates of STEC in deer meat might be less than roughly 1–2%.

In slaughterhouses for game meat in Japan, self-sanitation systems are needed to ensure excellent food hygiene, as laws concerning slaughter are not applied to these meats. Therefore on-site sanitary check systems should be required. Since the DNA strip method is rapid (within 60 min after DNA extraction), simple, and requires no special equipment, it will prove a promising method for on-site investigations in slaughterhouses.

Game meat, including deer meat, carries many different risk factors for food poisoning. *Sarcocystis* spp. is one such novel intrinsic risk factor for food poisoning [10]. We previously reported the presence of at least four species of *Sarcocystis* spp. in Japanese sika deer meat samples obtained from Yamanashi Prefecture, Japan [9], and designed our primers based on information in the 18S rRNA region of the predominant *Sarcocystis* spp*.,* among the 21 reported species. The results of cross-reaction experiment suggested that DNA strip for *Sarcocystis* spp. could be applied to other species of deer [8, 22] and *T. gondii*. However, whether or not the *stx1* and *stx2* gene primers used in this study collectively detect a series of *stx* variants is unclear. As deer-originating STEC can show variations in their *stx* genotypes [3, 5, 7, 11, 13], further studies will be required to clarify the above issues and expand the application of this detection method.

Despite these issues, the DNA strip approach developed in this study has several advantages, including its rapidity, multiplicity, and no need for special equipment, over other detection methods, such as conventional PCR, immunochromatography, and real-time PCR. Of further note, LAMP-lateral flow combined assays have also been recently developed for the detection of several other pathogenic microbes, such as *Toxoplasma* [17] and *Staphylococcus aureus* [25, 27].

In conclusion, our study showed that the “DNA strip” was able to detect *Sarcocystis* spp. and STEC in deer meat with high sensitivity. However, the improvement for the specificity of DNA strips is required to achieve specific reaction of the pathogens. Nevertheless, the application of this system will enable the quick and simple on-site inspection of food poisoning factors in deer meat.

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REFERENCES

1. Aoki, K., Ishikawa, K., Hayashi, K., Saito, M., Sugita-Konishi, Y., Watanabe, M. and Kamata, Y. 2013. An outbreak of suspected food poisoning related to deer meat containing *Sarcocystis* cysts. *Jpn. J. Food Microbiol.* **30**: 28–32. [CrossRef]
2. Asakura, H., Kawase, J., Ikeda, T., Honda, M., Sasaki, Y., Uema, M., Kabeya, H., Sugiyama, H., Igimi, S. and Takai, S. 2017. Microbiological quality assessment of game meats at retail in Japan. *J. Food Prot.* **80**: 2119–2126. [Medline] [CrossRef]
3. Asakura, H., Makino, S., Shirahata, T., Tsukamoto, T., Kurazono, H., Ikeda, T. and Takeshi, K. 1998. Detection and genetical characterization of Shiga toxin-producing *Escherichia coli* from wild deer. *Microbiol. Immunol.* **42**: 815–822 . [Medline] [CrossRef]
4. Cong, W., Qin, S. Y., Meng, Q. F., Zou, F. C., Qian, A. D. and Zhu, X. Q. 2016. Molecular detection and genetic characterization of *Toxoplasma gondii* infection in sika deer (*Cervus nippon*) in China. *Infect. Genet. Evol.* **39**: 9–11 . [Medline] [CrossRef]
5. Díaz-Sánchez, S., Sánchez, S., Herrera-León, S., Porrero, C., Blanco, J., Dahbi, G., Blanco, J. E., Mora, A., Mateo, R., Hanning, I. and Vidal, D. 2013. Prevalence of Shiga toxin-producing *Escherichia coli, Salmonella* spp. and *Campylobacter* spp. in large game animals intended for consumption: relationship with management practices and livestock influence. *Vet. Microbiol.* **163**: 274–281 . [Medline] [CrossRef]
6. Dong, H. J., Cho, A. R., Hahn, T. W. and Cho, S. 2014. Development of a multiplex loop-mediated isothermal amplification assay to detect shiga toxin-producing *Escherichia coli* in cattle. *J. Vet. Sci.* **15**: 317–325 . [Medline] [CrossRef]
7. Eggert, M., Stüber, E., Heurich, M., Fredriksson-Ahomaa, M., Burgos, Y., Beutin, L. and Märtlbauer, E. 2013. Detection and characterization of Shiga toxin-producing *Escherichia coli* in faeces and lymphatic tissue of free-ranging deer. *Epidemiol. Infect.* **141**: 251–259 . [Medline] [CrossRef]
8. Gjerde, B., Vikøren, T. and Hamnes, I. S. 2017. Molecular identification of *Sarcocystis halieti* n. sp., *Sarcocystis lari* and *Sarcocystis truncata* in the intestine of a white-tailed sea eagle (*Haliaeetus albicilla*) in Norway. *Int. J. Parasitol. Parasites Wildl.* **7**: 1–11 . [Medline] [CrossRef]
9. Honda, M., Sawaya, M., Taira, K., Yamazaki, A., Kamata, Y., Shimizu, H., Kobayashi, N., Sakata, R., Asakura, H. and Sugita-Konishi, Y. 2018. Effects of temperature, pH and curing on the viability of *Sarcocystis,* a Japanese sika deer (*Cervus Nippon centralis*) parasite, and the inactivation of their diarrheal toxin. *J. Vet. Med. Sci.* **80**: 1337–1344 . [Medline] [CrossRef]
10. Irikura, D., Saito, M., Sugita-Konishi, Y., Ohnishi, T., Sugiyama, K. I., Watanabe, M., Yamazaki, A., Izumiyama, S., Sato, H., Kimura, Y., Doi, R. and Kamata, Y. 2017. Characterization of Sarcocystis fayeri’s actin-depolymerizing factor as a toxin that causes diarrhea. *Genes Cells* **22**: 825–835 . [Medline] [CrossRef]
11. Ishii, S., Meyer, K. P. and Sadowsky, M. J. 2007. Relationship between phylogenetic groups, genotypic clusters, and virulence gene profiles of *Escherichia coli* strains from diverse human and animal sources. *Appl. Environ. Microbiol.* **73**: 5703–5710 . [Medline] [CrossRef]
12. Japan Ministry of Environments 2014. Protection and control of wild birds and mammals and hunting management act. http://elaws.e-gov.go.jp/search/elawsSearch/elaws_search/lsg0500/detail?lawId=414AC0000000088&openerCode=1 [accessed on July 1, 2018].
13. Kabeya, H., Sato, S., Oda, S., Kawamura, M., Nagasaka, M., Kuranaga, M., Yokoyama, E., Hirai, S., Iguchi, A., Ishihara, T., Kuroki, T., Morita-

Ishihara, T., Iyoda, S., Terajima, J., Ohnishi, M. and Maruyama, S. 2017. Characterization of Shiga toxin-producing *Escherichia coli* from feces of sika deer (*Cervus nippon*) in Japan using PCR binary typing analysis to evaluate their potential human pathogenicity. *J. Vet. Med. Sci.* **79**: 834–841 . [Medline] [CrossRef]

14. Kamata, Y., Saito, M., Irikura, D., Yahata, Y., Ohnishi, T., Bessho, T., Inui, T., Watanabe, M. and Sugita-Konishi, Y. 2014. A toxin isolated from *Sarcocystis fayeri* in raw horsemeat may be responsible for food poisoning. *J. Food Prot.* **77**: 814–819 . [Medline] [CrossRef]
15. Keene, W. E., Sazie, E., Kok, J., Rice, D. H., Hancock, D. D., Balan, V. K., Zhao, T. and Doyle, M. P. 1997. An outbreak of *Escherichia coli* O157:H7 infections traced to jerky made from deer meat. *JAMA* **277**: 1229–1231 . [Medline] [CrossRef]
16. Kim, C. S., Kim, D. S. and Jung, H. R. 2019. Toxoplasma lymphadenitis caused by ingestion of raw blood and meat of deer in a 10-year-old boy. *Pediatr. Neonatol.* **60**: 112–113 . [Medline] [CrossRef]
17. Lalle, M., Possenti, A., Dubey, J. P. and Pozio, E. 2018. Loop-mediated isothermal amplification-lateral-flow dipstick (LAMP-LFD) to detect toxoplasma gondii oocyst in ready-to-eat salad. *Food Microbiol.* **70**: 137–142 . [Medline] [CrossRef]
18. Li, Y., Fan, P., Zhou, S. and Zhang, L. 2017. Loop-mediated isothermal amplification (LAMP): A novel rapid detection platform for pathogens. *Microb. Pathog.* **107**: 54–61 . [Medline] [CrossRef]
19. Nielsen, E. M. and Andersen, M. T. 2003. Detection and characterization of verocytotoxin-producing *Escherichia coli* by automated 5′ nuclease PCR assay. *J. Clin. Microbiol.* **41**: 2884–2893 . [Medline] [CrossRef]
20. Pritt, B., Trainer, T., Simmons-Arnold, L., Evans, M., Dunams, D. and Rosenthal, B. M. 2008. Detection of *sarcocystis* parasites in retail beef: a regional survey combining histological and genetic detection methods. *J. Food Prot.* **71**: 2144–2147 . [Medline] [CrossRef]
21. Priyanka, B., Patil, R. K. and Dwarakanath, S. 2016. A review on detection methods used for foodborne pathogens. *Indian J. Med. Res.* **144**: 327–338 . [Medline] [CrossRef]
22. Rudaitytė-Lukošienė, E., Prakas, P., Butkauskas, D., Kutkienė, L., Vepštaitė-Monstavičė, I. and Servienė, E. 2018. Morphological and molecular identification of *Sarcocystis* spp. from the sika deer (*Cervus nippon*), including two new species *Sarcocystis frondea* and *Sarcocystis nipponi*. *Parasitol. Res.* **117**: 1305–1315 . [Medline] [CrossRef]
23. Takasaki, K., Yamakoshi, Y. and Futo, S. 2018. Single-laboratory validation of rapid and easy DNA strip for porcine DNA detection in beef meatballs. *J. AOAC Int.* **101**: 1653–1656 . [Medline] [CrossRef]
24. Tei, S., Kitajima, N., Takahashi, K. and Mishiro, S. 2003. Zoonotic transmission of hepatitis E virus from deer to human beings. *Lancet* **362**: 371–373 . [Medline] [CrossRef]
25. Wang, Y., Li, H., Wang, Y., Zhang, L., Xu, J. and Ye, C. 2017. Loop-mediated isothermal amplification label-based gold nanoparticles lateral flow biosensor for detection of *Enterococcus faecalis* and *Staphylococcus aureus*. *Front. Microbiol.* **8**: 192. [Medline] [CrossRef]
26. Yamazaki, A., Honda, M., Kobayashi, N., Ishizaki, N., Asakura, H. and Sugita-Konishi, Y. 2018. The sensitivity of commercial kits in detecting the genes of pathogenic bacteria in venison. *J. Vet. Med. Sci.* **80**: 706–709 . [Medline] [CrossRef]
27. Yin, H. Y., Fang, T. J. and Wen, H. W. 2016. Combined multiplex loop-mediated isothermal amplification with lateral flow assay to detect *sea* and *seb* genes of enterotoxic *Staphylococcus aureus*. *Lett. Appl. Microbiol.* **63**: 16–24 . [Medline] [CrossRef]
28. Zhao, X., Lin, C. W., Wang, J. and Oh, D. H. 2014. Advances in rapid detection methods for foodborne pathogens. *J. Microbiol. Biotechnol.* **24**: 297–312 . [Medline] [CrossRef]