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Development of loop-mediated isothermal amplification to detect *Streptococcus suis* and its application to retail pork meat in Japan

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ABSTRACT

We here developed a novel loop-mediated isothermal amplification (LAMP) method to detect *Streptococcus suis* in raw pork meat. This method, designated $LAMP_{SS}$, targeted the recombination/repair protein (*recN*) gene of *S. suis* and detected all serotypes of *S. suis*, except those taxonomically removed from authentic *S. suis*, i.e., serotypes 20, 22, 26, 32, 33, and 34. The specificity of $LAMP_{SS}$ was confirmed and its detection limit was 5.4 cfu/reaction. Among the 966 raw pork meat samples examined, including sliced pork, minced pork, and the liver, tongue, heart, and small intestine, 255 samples tested positive with $LAMP_{SS}$. The rate of contamination was higher in the organs than in pork. No significant difference was observed in the total bacterial count between $LAMP_{SS}$-positive and -negative samples. The number of shops that provided $LAMP_{SS}$-positive pork was slightly higher in those that sold swine organs and pork than in those that sold only pork, suggesting that cross contamination occurred from the organs to pork. Among the 255 which tested positive for $LAMP_{SS}$, only 47 samples tested positive for the previously described LAMP specific for *S. suis* serotype 2. Two isolates of *S. suis* serotype 2, belonging to sequence type 28, which is potentially hazardous to humans, as well as those of some other serotypes were obtained from 19 out of 47 samples by combining LAMP with a replica plating method. These results suggest that $LAMP_{SS}$ will be a useful tool for the surveillance of raw pork meat in the retail market.

1. Introduction

Streptococcus suis is a zoonotic pathogen that causes serious diseases, including meningitis, arthritis, septicemia, and endocarditis in pigs and humans, and is responsible for severe economic losses in the swine industry worldwide (Goyette-Desjardins et al., 2014). In Western countries, *S. suis* infections in humans have generally been restricted to workers in close contact with pigs or swine by-products. However, in East and Southeast Asia, *S. suis* represents a significant public health concern (Goyette-Desjardins et al., 2014), as this bacterium was identified as the first, second, and third causes of adult meningitis in Viet Nam, Thailand, and Hong Kong, respectively (Ip et al., 2007; Mai et al., 2008; Suankratay et al., 2004). In Viet Nam and Thailand, some people consume undercooked pork including organs, resulting in many patients having *S. suis* infection described above (Nghia et al., 2011; Pachirat et al., 2012). Furthermore, *S. suis* was isolated from or detected in raw pork meat sold in markets in Hong Kong (Cheung et al., 2008; Ip et al., 2007). This finding suggests that raw pork meat can be a source of human infection and the threat of *S. suis* to food safety needs to be recognized.

To date, 35 serotypes of *S. suis* (serotypes 1–34 and serotype 1/2) have been described on the basis of their polysaccharide capsular antigens (Goyette-Desjardins et al., 2014). However, phylogenetic analyses of the 16S rRNA and chaperonin-60 (*cpn*60) genes showed that the reference strains of serotypes 32 and 34 should have been *Streptococcus orisratti* (Hill et al., 2005). More recent sequence analyses of genes encoding manganese-dependent superoxide dismutase (*sodA*) and the recombination/repair protein (*recN*) indicated that the reference strains of serotypes 20, 22, 26, and 33 need to be taxonomically removed from *S. suis* (Tien le et al., 2013), and serotypes 20, 22, and 26 was reappraised and proposed to be a novel species, *Streptococcus parasuis* (Nomoto et al., 2015).

Notomi et al. (2000) developed a nucleic acid amplification method called loop-mediated isothermal amplification (LAMP), the reaction which is performed under isothermal conditions with a set of four to six specially designed primers and can be accomplished in a single step without the need for advanced instruments (Mori and Notomi,

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Table 1
Bacteria strains and isolates used in this study and $LAMP_{SS}$ results.

Species	Strains	Serotype of *S. suis*	Source	$LAMP_{SS}$
Streptococcus suis	Type strain and serotype reference strains			
	NCTC 10237 (=5428)	1	Diseased pig	+
	NCTC 10234^T (=S735)	2	Diseased pig	+
	4961	3	Diseased pig	+
	6407	4	Diseased pig	+
	11538	5	Diseased pig	+
	2524	6	Diseased pig	+
	8074	7	Diseased pig	+
	14636	8	Diseased pig	+
	22083	9	Diseased pig	+
	4417	10	Diseased pig	+
	12814	11	Diseased pig	+
	8830	12	Diseased pig	+
	10581	13	Diseased pig	+
	13730	14	Diseased human	+
	NCTC 10446	15	Diseased pig	+
	2726	16	Diseased pig	+
	93A	17	Clinically healthy pig	+
	NT77	18	Clinically healthy pig	+
	42A	19	Clinically healthy pig	+
	86-5192	20	Diseased calf	−
	14A	21	Clinically healthy pig	+
	88-1861	22	Diseased pig	−
	89-2479	23	Diseased pig	+
	88-5299A	24	Diseased pig	+
	89-3576-3	25	Diseased pig	+
	89-4109-1	26	Diseased pig	−
	89-5259	27	Diseased pig	+
	89-590	28	Diseased pig	+
	92-1191	29	Diseased pig	+
	92-1400	30	Diseased pig	+
	92-4172	31	Diseased calf	+
	EA1172.91	32	Diseased pig	−
	EA1832.92	33	Diseased lamb	−
	92-2742	34	Diseased pig	−
	2651	1/2	Diseased pig	+
	Representative strains, human isolate, and porcine isolates			
	P1/7	2	Diseased pig	+
	89-1591	2	Diseased pig	+
	GUT-49	1	Diseased pig	+
	GUT-6	2	Diseased pig	+
	GUT-14	3	Diseased pig	+
	GUT-1	4	Diseased pig	+
	GUT-58	5	Diseased pig	+
	GUT-8	7	Diseased pig	+
	GUT-27	8	Diseased pig	+
	GUT-7	9	Diseased pig	+
	GUT-156	11	Diseased pig	+
	SUT-38	12	Clinically healthy pig	+
	GUT-32	15	Diseased pig	+
	SUT-246	16	Clinically healthy pig	+
	SUT-283	20	Clinically healthy pig	−
	SUT-380	22	Clinically healthy pig	−
	GUT-33	25	Diseased pig	+
	GUT-35	31	Diseased pig	+
	GUT-183	33	Diseased calf	−
Streptococcus acidominimus	ATCC 51725^T		a	−
Streptococcus dysgalactiae subsp. *equisimilis*	ATCC 35666		b	−
Streptococcus entericus	JCM 12180^T		c	−
Streptococcus gallinaceus	CCUG 42692^T		a	−
Streptococcus minor	CCUG 47487^T		a	−
Streptococcus oralis	JCM 12997^T		c	−
Streptococcus orisratti	ATCC 700640^T		a	−
Streptococcus ovis	CCUG 39485^T		a	−
Streptococcus pluranimalium	FKI2012		d	−
Streptococcus plurextorum	CECT 7308^T		e	−
Streptococcus porci	CECT 7374^T		e	−
Streptococcus porcinus	ATCC 43138^T		b	−
Streptococcus pyogenes	ATCC 12344^T		f	−
Actinobacillus pleuropneumoniae	FBPM-460		g	−
Bordetella bronchiseptica	FBPM-462		h	−
Brachyspira hyodysenteriae	ATCC 27164^T		i	−
Erysipelothrix rhusiopathiae	Fujisawa		j	−
Erysipelothrix tonsillarum	ATCC 43339^T		j	−
Escherichia coli	MC-1		a, k	−