

Current Status of Human Taeniasis in Lao People's Democratic Republic

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Abstract: Human taeniasis was investigated in Lao People's Democratic Republic (Lao PDR) between 2000 and 2011 as part of the nation's helminthiasis survey. A total of 55,038 inhabitants, including 29,846 school children, were examined using the Kato-Katz and scotch-tape anal swab method, and morphological observation of adult worms. Molecular identification of *Taenia* tapeworms was performed by multiplex PCR or DNA sequence analysis of the mitochondrial *cox1* gene. *Taenia* eggs were present at a rate of 1.5% (845/55,038) in the subject population. Adult tapeworms were identified as *T. solium* or *T. saginata* by analyzing the collectable stool specimens (n = 126). Three specimens identified as *T. solium* were found in Luang Prabang, while the remaining 123 specimens, which were *T. saginata*, were found in Bokeo, Bolikhamxay, Champasak, Houaphan, Khammouane, Luang Namta, Luang Prabang, Oudomxay, Phongsaly, Saysomboune, Saravane, Savannakhet, Xayaboury, Xekong, Xieng Khouang Province, and Vientiane Municipality.

Key words: *Taenia saginata*, *Taenia solium*, human taeniasis, Lao PDR

Taenia solium, *T. saginata*, and *T. asiatica* are taeniid tapeworms that cause taeniasis in humans and cysticercosis in intermediate host animals. Taeniasis and cysticercosis remain a significant public health problem in regions of Asia, Africa, Eastern Europe, and Central and South America. Information on the distribution and prevalence of taeniasis and cysticercosis in Indochina (Cambodia, Lao PDR, and Vietnam) is rather limited when compared to that for endemic regions of Latin America and Africa.

Prevalence data on taeniasis indicated that *T. solium* is more common than *T. saginata* in Lao PDR [1]. Studies on human taeniasis in Lao PDR have been carried out in Vientiane, Khammouane, and Champasak Province between 1989 and

2002 [2-5]. In addition, a nationwide survey was conducted between 2000 and 2002 using cellophane anal swab and the Kato-Katz method [6]. In 2004, a total of 814 subjects from 13 villages in Saravane Province were investigated, and the overall prevalence of taeniasis in the population was shown to be 5% [7]. These data were confirmed by coprological egg examination or morphological observations of the expelled proglottids. However, for Lao PDR, so far very little information is available on differential *Taenia* species identification despite having conducted surveys.

Epidemiologically, all 3 *Taenia* tapeworms that infect humans have been found in Asian countries, including China, Indonesia, Korea, the Philippines, Taiwan, Thailand, Vietnam, and Japan [8]. However, the epidemiological distribution status of these tapeworms still needs to be clarified in Lao PDR.

International collaboration projects between Korea and Lao PDR have been ongoing since 2000. These 11-year nationwide surveys on 17 provinces and Vientiane Municipality involved stool examination using the Kato-Katz and scotch-tape anal

•Received 18 October 2012, revised 14 November 2012, accepted 1 December 2012.

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swab method. The aim of these projects was to eliminate helminth infections and promote health by controlling these infections and appropriately treating them. The present study focused on identification of *Taenia* cases in Lao PDR by microscopic examinations for the presence of eggs in stool, morphological observation of adult tapeworms, multiplex PCR, and nucleotide analysis for sequence variation.

A total of 55,038 fecal specimens were collected between 2000 and 2011 from 17 provinces of Lao PDR, including 176 districts of 18 regions. A nationwide survey on 29,846 children aged 6-11 years, from 317 primary schools, was conducted between 2000 and 2002. The remaining fecal specimens (n=25,192) were collected from schoolchildren and residents between 2002 and 2011. The Vientiane Municipality (n=599), Savannakhet (n=981), Champasak (n=620), Attapeu (n=763), Luang Prabang (n=589) and Xayaboury (n=558) were surveyed in 2002; Khammouane (n=1,242) and Saravane (n=1,270) were surveyed in 2003; and Vientiane Municipality (n=185), Luang Namta (n=198), and Savannakhet (n=248) were surveyed in 2005. A nationwide survey on 11,770 schoolchildren aged 6-11 years was also conducted in 2005. The Vientiane Municipality (n=527) was surveyed in December, 2007; Savannakhet and Phongsaly Province (n=11,115) in July and December, 2008; Khammouane Province (n=5,247) was surveyed in June, 2009; Saravane Province (n=4,586) was surveyed in December, 2009; Champasak Province (n=669) was surveyed in June, 2010; Champasak Province and Vientiane Municipality (n=426) were surveyed in December, 2010; Xieng Khouang (n=642) was surveyed in June, 2011; and Luang Prabang (n=557) was surveyed in December, 2011 (Table 1).

Fecal specimens were examined for intestinal helminth eggs using the Kato-Katz thick smear technique. A total of 126 *Taenia* tapeworms were recovered from patients who were positive for intestinal helminth eggs. Patients discharged the adult tapeworms after treatment with a single oral dose of 20-30 mg praziquantel (Shinpoong Pharm. Co., Seoul, Korea) followed by purgation with magnesium salts. The worms were fixed in 10% formalin under slide glass pressure and then acetocarmine-stained for morphological identification using a light microscope. Some worms were fixed in 70% ethanol for genetic identification by DNA sequencing.

The purified PCR-amplified fragments of the *cox1* gene were directly sequenced. The primer walking method was employed to obtain direct sequences for each of the amplified fragments. Cyclic sequencing from both ends of the fragments was per-

formed using a Big-Dye Terminator sequencing kit (Applied Biosystems, Foster City, California, USA) and the reaction products were electrophoresed on an automated DNA sequencer (model 3730KL, Applied Biosystems). The sequences were assembled and aligned using the Bioedit program version 5.0.6 (BIOSOFT, Ferguson, Missouri, USA). Using BLAST searches, the sequenced regions were identified by comparing them with those of *Taenia* tapeworms, which had been deposited in the GenBank database. We identified *Taenia* tapeworm specimens by comparing the nucleotide sequences obtained with those of the *cox1* genes of *T. solium* (GenBank no. AB086256), *T. saginata* (Genbank no. AY684274), and *T. asiatica* (GenBank no. AF445798). Multiplex PCR was performed on all specimens as described previously [9].

The tapeworm specimens (n=126) collected were analyzed by observing their morphological features and nucleotide sequences of the mitochondrial *cox1* gene. Adult tapeworms and metacestodes were examined morphologically after carmine staining. Genomic DNA of the specimens was extracted with the DNeasy Tissue Kit (Qiagen, Valencia, California, USA). The PCR amplification products were directly sequenced using the

Table 1. Prevalence of taeniasis in 17 provinces and the Vientiane Municipality, Lao PDR (2000–2011)

Locality (Province)	No. of people examined	No. of positive (%)
Vientiane Municipality ^{a,b,c,d}	4,626	75 (1.6)
Vientiane ^{a,c,d}	2,382	7 (0.2)
Savannakhet ^{a,b,c}	7,371	240 (3.2)
Bolikhamxay ^{a,c}	2,596	9 (0.3)
Saravane ^{a,b,c,d}	4,118	128 (3.1)
Khammouane ^{a,b,c,d}	4,341	145 (3.3)
Champasak ^{a,b,c,d}	4,043	60 (1.4)
Xekong ^{a,c}	1,713	30 (1.7)
Attapeu ^{a,b,c}	2,123	9 (0.4)
Luang Prabang ^{a,b,c,d}	4,059	54 (1.3)
Oudomxay ^{a,c}	2,627	40 (1.5)
Xayaboury ^{a,b,c}	2,951	10 (0.3)
Houaphan ^a	1,431	17 (1.1)
Louang Namtha ^{a,b,c}	2,435	2 (0.1)
Saysombune ^a	1,283	1 (0.1)
Phongsaly ^{a,c,d}	2,133	3 (0.1)
Bokeo ^{a,c}	2,113	3 (0.1)
Xieng Khouang ^{a,c,d}	2,693	12 (0.4)
Total	55,038	845 (1.5)

Fecal examination was performed by the Kato-Katz method.

^a2000-2002: School children (n=29,846); ^b2002-2004: Inhabitants of 16 Provinces over 15 years of age (n=7,244); ^c2005: Nationwide survey on school children aged 6-11 years (n=11,770); ^d2007-2011: Inhabitants of 7 Provinces over 15 years of age (n=6,178).

Big-Dye Terminator Cycle Sequencing Kit (Applied Biosystems).

A total of 55,038 inhabitants, including 29,846 schoolchildren, were examined by coprological microscopy between 2000 and 2011. The prevalence of *Taenia* eggs was 1.5% (845/55,038) (Table 1). A total of 126 adult tapeworms were collected in Savannakhet, Saravane, Oudomxay, Luang Prabang, Champasak, Xieng Khouang, and Vientiane. Other isolated helminth eggs included *Ascaris lumbricoides*, hookworms, *Trichuris trichiura*, *Trichostrongylus orientalis*, *Opisthorchis viverrini*/minute intestinal flukes, and *Echinostoma* spp. A slightly higher prevalence of human taeniasis (3.1% and 3.3%) was observed in Savannakhet, Saravane, and Khammouane, respectively. In the remaining provinces, the prevalence of taeniasis was 0.7% on average (Fig. 1), with the highest (3.3%) in Khammouane.

Molecular identification of *Taenia* tapeworms was performed by multiplex PCR and DNA sequence analysis, corresponding to positions 90-530 of the *cox1* gene. The *cox1* sequences (440 bp) with code numbers 1678, 1685, and 1687 showed 99.7% similarity with the reference sequence of *T. solium* (GenBank no. AB086256), and the remaining samples showed 99.3% similarity with *T. saginata* (GenBank no. AY684274). Multiplex PCR revealed a 474 bp diagnostic band in the *T. solium* samples with code numbers 1678, 1685, and 1687. The remaining 123 specimens were identified by DNA sequencing of the *cox1* gene and multiplex PCR, revealing a 629 bp diagnostic band specific to *T. saginata* (Table 2). The *Taenia* tapeworms isolated

from Lao PDR were *T. solium* and *T. saginata*. Three *T. solium* specimens were found in Luang Prabang, and 123 *T. saginata* specimens were found in other provinces (Table 2).

Numerous surveys on intestinal parasite infections have been conducted in Lao PDR over the past 20 years, including 9 published papers. However, information on taeniasis and cysticercosis from community-based surveys in Lao PDR is rather limited when compared to information from endemic regions in other Asian countries and Latin America. The present study was carried out between 2000 and 2011 on a total of 55,038 fecal specimens, including 126 adult tapeworms collected from 176 districts in 18 regions including 17 provinces of Lao PDR. The prevalence of human taeniasis ranged from 0.1% to 3.3%. Central regions such as Saravane, Savannakhet, and Khammouane showed higher prevalence of human taeniasis. A previous nationwide survey of human taeniasis among primary schoolchildren observed a 0.6% incidence [6]. Another study on human taeniasis indicated that all 3 species infecting humans were present in Lao PDR [10]. Further studies are required to confirm this. In addition, previous studies have shown that *T. solium* is more common than *T. saginata* in this area [1]. This is in disagreement with our results, which show that *T. saginata* is more common than *T. solium*. One of the sources of infection for *T. saginata* is believed to be “lap”, a Laotian dish made of raw cow’s liver, commonly consumed in these communities. Risk factors for *T. solium* taeniasis and cysticercosis infection in Lao PDR may come from consuming raw pork such as “sommou” and using human feces to fertilize vegetable gardens. The habit of eating raw pork is quite common in northern highlands in Vietnam [11] and also in Lao PDR.

Previously, clear reports on *T. solium* adult tapeworms were almost not available. In the present study, through morphological and genetic analysis, we identified 3 specimens of *T. solium* out of 126 collected worms. The *T. solium* infections observed came from inhabitants of the Lathahea district, Luang Prabang, which is located in the northern part of Lao PDR. In addition, a male patient from Oudomxay, a province near our survey region, was diagnosed with neurocysticercosis in 2005. He had been previously diagnosed by CT scan in Thailand, following an epileptic seizure in 1993. In 2007, a pig with a heavy *T. solium* metacestode infection was discovered in a slaughterhouse in Oudomxay (unpublished observation).

All 3 species of human *Taenia* tapeworms, *T. solium*, *T. saginata*, and *T. asiatica* have been found in countries near Lao PDR, like Vietnam, Thailand, and Yunnan Province of China

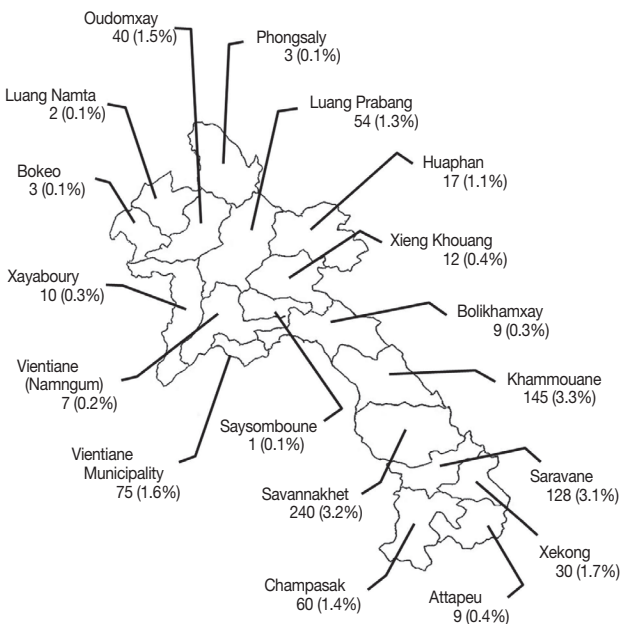


Fig. 1. Surveyed provinces and districts in Lao PDR.

Table 2. *Taenia* tapeworm specimens of Laotians analyzed in the present study (2002-2011)

Code	Location	Sex	Age	Year	cox 1 sequence and multiplex PCR finding	Code	Location	Sex	Age	Year	cox 1 sequence and multiplex PCR finding
1310	Savannakhet	F	48	2002.07	<i>T. saginata</i>	1645	Luang Prabang	-	48	2007.06	<i>T. saginata</i>
1311	Savannakhet	F	52	2002.07	<i>T. saginata</i>	1646	Luang Prabang	-	38	2007.06	<i>T. saginata</i>
1312	Savannakhet	M	60	2002.07	<i>T. saginata</i>	1647	Luang Prabang	-	62	2007.06	<i>T. saginata</i>
1313	Savannakhet	F	29	2002.07	<i>T. saginata</i>	1678	Luang Prabang	M	-	2007.12	<i>T. solium</i>
1314	Savannakhet	F	31	2002.07	<i>T. saginata</i>	1713	Luang Prabang	M	29	2007.12	<i>T. saginata</i>
1315	Savannakhet	F	55	2002.07	<i>T. saginata</i>	1714	Luang Prabang	M	-	2007.12	<i>T. saginata</i>
1316	Savannakhet	F	55	2002.07	<i>T. saginata</i>	1715	Luang Prabang	F	35	2007.12	<i>T. saginata</i>
1317	Savannakhet	F	58	2002.07	<i>T. saginata</i>	1716	Luang Prabang	M	60	2007.12	<i>T. saginata</i>
1318	Savannakhet	F	58	2002.07	<i>T. saginata</i>	1717	Luang Prabang	F	65	2007.12	<i>T. saginata</i>
1319	Savannakhet	F	42	2002.07	<i>T. saginata</i>	1718	Luang Prabang	M	28	2007.12	<i>T. saginata</i>
1320	Savannakhet	F	69	2002.07	<i>T. saginata</i>	1719	Luang Prabang	M	40	2007.12	<i>T. saginata</i>
1321	Savannakhet	F	32	2002.07	<i>T. saginata</i>	1720	Luang Prabang	M	59	2007.12	<i>T. saginata</i>
1322	Savannakhet	F	37	2002.07	<i>T. saginata</i>	1721	Luang Prabang	F	12	2007.12	<i>T. saginata</i>
1417	Saravane	M	16	2003.11	<i>T. saginata</i>	1722	Luang Prabang	M	52	2007.12	<i>T. saginata</i>
1418	Saravane	F	26	2003.11	<i>T. saginata</i>	1723	Luang Prabang	F	88	2007.12	<i>T. saginata</i>
1419	Saravane	F	50	2003.11	<i>T. saginata</i>	1719	Vientiane	F	48	2007.12	<i>T. saginata</i>
1420	Saravane	F	48	2003.11	<i>T. saginata</i>	1720	Vientiane	F	47	2007.12	<i>T. saginata</i>
1421	Saravane	F	32	2003.11	<i>T. saginata</i>	1721	Vientiane	F	70	2007.12	<i>T. saginata</i>
1422	Saravane	F	27	2003.11	<i>T. saginata</i>	1722	Vientiane	F	63	2007.12	<i>T. saginata</i>
1423	Saravane	M	30	2003.11	<i>T. saginata</i>	1723	Vientiane	F	52	2007.12	<i>T. saginata</i>
1424	Saravane	M	48	2003.11	<i>T. saginata</i>	1724	Vientiane	M	63	2007.12	<i>T. saginata</i>
1425	Saravane	F	38	2003.11	<i>T. saginata</i>	1784	Luang Prabang	F	70	2008.06	<i>T. saginata</i>
1441	Savannakhet	F	11	2004.02	<i>T. saginata</i>	1685	Luang Prabang	F	50	2008.06	<i>T. solium</i>
1442	Savannakhet	M	11	2004.02	<i>T. saginata</i>	1725	Luang Prabang	M	64	2008.06	<i>T. saginata</i>
1443	Savannakhet	M	12	2004.02	<i>T. saginata</i>	1726	Luang Prabang	M	60	2008.06	<i>T. saginata</i>
1444	Savannakhet	M	13	2004.02	<i>T. saginata</i>	1727	Luang Prabang	M	70	2008.06	<i>T. saginata</i>
1445	Savannakhet	M	13	2004.02	<i>T. saginata</i>	1728	Luang Prabang	M	20	2008.06	<i>T. saginata</i>
1446	Savannakhet	M	13	2004.02	<i>T. saginata</i>	1729	Luang Prabang	M	65	2008.06	<i>T. saginata</i>
1447	Savannakhet	M	13	2004.02	<i>T. saginata</i>	1730	Luang Prabang	M	40	2008.06	<i>T. saginata</i>
1448	Savannakhet	M	14	2004.02	<i>T. saginata</i>	1687	Luang Prabang	M	45	2008.06	<i>T. solium</i>
1449	Savannakhet	M	12	2004.02	<i>T. saginata</i>	1731	Luang Prabang	M	68	2008.06	<i>T. saginata</i>
1450	Savannakhet	M	12	2004.02	<i>T. saginata</i>	1732	Luang Prabang	F	50	2008.06	<i>T. saginata</i>
1451	Savannakhet	M	28	2004.02	<i>T. saginata</i>	1733	Savannakhet	M	67	2008.06	<i>T. saginata</i>
1452	Savannakhet	F	35	2004.02	<i>T. saginata</i>	1734	Savannakhet	F	59	2008.06	<i>T. saginata</i>
1453	Savannakhet	F	60	2004.02	<i>T. saginata</i>	1735	Savannakhet	F	48	2008.06	<i>T. saginata</i>
1454	Savannakhet	M	53	2004.02	<i>T. saginata</i>	1736	Savannakhet	M	56	2008.06	<i>T. saginata</i>
1455	Savannakhet	F	38	2004.02	<i>T. saginata</i>	1737	Savannakhet	M	40	2008.06	<i>T. saginata</i>
1456	Savannakhet	M	34	2004.02	<i>T. saginata</i>	1738	Savannakhet	M	47	2008.06	<i>T. saginata</i>
1457	Savannakhet	F	43	2004.02	<i>T. saginata</i>	1739	Savannakhet	-	-	2008.12	<i>T. saginata</i>
1458	Savannakhet	M	40	2004.02	<i>T. saginata</i>	1740	Savannakhet	-	-	2008.12	<i>T. saginata</i>
1597	Oudomxay	M	53	2005.11	<i>T. saginata</i>	1741	Phongsaly	M	54	2008.12	<i>T. saginata</i>
1598	Oudomxay	M	62	2005.11	<i>T. saginata</i>	1742	Saravane	-	-	2009.12	<i>T. saginata</i>
1599	Oudomxay	M	65	2005.11	<i>T. saginata</i>	1743	Saravane	-	-	2009.12	<i>T. saginata</i>
1600	Oudomxay	M	55	2005.11	<i>T. saginata</i>	1744	Saravane	-	-	2009.12	<i>T. saginata</i>
1601	Oudomxay	M	50	2005.11	<i>T. saginata</i>	1745	Champasak	F	35	2010.06	<i>T. saginata</i>
1602	Oudomxay	M	41	2005.11	<i>T. saginata</i>	1746	Champasak	-	-	2010.06	<i>T. saginata</i>
1603	Oudomxay	M	35	2005.11	<i>T. saginata</i>	1747	Champasak	-	-	2010.06	<i>T. saginata</i>
1604	Oudomxay	M	35	2005.11	<i>T. saginata</i>	1748	Champasak	-	-	2010.12	<i>T. saginata</i>
1605	Oudomxay	M	27	2005.11	<i>T. saginata</i>	1749	Champasak	-	-	2010.12	<i>T. saginata</i>
1606	Oudomxay	M	53	2005.11	<i>T. saginata</i>	1750	Champasak	-	-	2010.12	<i>T. saginata</i>
1607	Oudomxay	M	22	2005.11	<i>T. saginata</i>	1751	Champasak	M	55	2010.12	<i>T. saginata</i>
1608	Oudomxay	M	32	2005.11	<i>T. saginata</i>	1752	Xieng Khouang	-	-	2011.06	<i>T. saginata</i>
1609	Oudomxay	M	24	2005.11	<i>T. saginata</i>	1753	Xieng Khouang	-	-	2011.06	<i>T. saginata</i>
1610	Oudomxay	M	65	2005.11	<i>T. saginata</i>	1754	Xieng Khouang	-	-	2011.06	<i>T. saginata</i>
1611	Oudomxay	M	47	2005.11	<i>T. saginata</i>	1755	Xieng Khouang	-	-	2011.06	<i>T. saginata</i>
1612	Oudomxay	F	32	2005.11	<i>T. saginata</i>	1756	Xieng Khouang	-	-	2011.06	<i>T. saginata</i>
1613	Oudomxay	M	40	2005.11	<i>T. saginata</i>	1757	Xieng Khouang	-	-	2011.06	<i>T. saginata</i>
1639	Luang Prabang	-	37	2007.06	<i>T. saginata</i>	1758	Luang Prabang	-	-	2011.12	<i>T. saginata</i>
1640	Luang Prabang	-	25	2007.06	<i>T. saginata</i>	1759	Luang Prabang	-	-	2011.12	<i>T. saginata</i>
1641	Luang Prabang	-	35	2007.06	<i>T. saginata</i>	1760	Luang Prabang	-	30	2011.12	<i>T. saginata</i>
1642	Luang Prabang	-	28	2007.06	<i>T. saginata</i>	1761	Luang Prabang	-	40	2011.12	<i>T. saginata</i>
1643	Luang Prabang	-	45	2007.06	<i>T. saginata</i>	1762	Luang Prabang	-	60	2011.12	<i>T. saginata</i>
1644	Luang Prabang	-	-	2007.06	<i>T. saginata</i>	1763	Luang Prabang	-	50	2011.12	<i>T. saginata</i>

[12]. *T. asiatica* has been found in many Asian countries, such as Korea, China, Indonesia, the Philippines, Vietnam, Thailand, and Japan; however, its distribution in Lao PDR still needs to be clarified. Recently, molecular diagnostic methods have been developed for the rapid and accurate detection of tapeworm infections, including the use of formalin-fixed or sectioned and stained specimens. If more molecular diagnostic methods are applied to field surveys, it would be possible to obtain more precise epidemiological data on tapeworm infections in Lao PDR.

ACKNOWLEDGMENTS

We thank the staff of the Center for Laboratory and Epidemiology, Department of Hygiene and Prevention, Vientiane, and Provincial Health Department, Lao PDR for their effort in collecting fecal samples and preparing Kato-Katz smears. The Korea Association of Health Promotion (KAHP) and the Korea International Cooperation Agency (KOICA) have supported intestinal parasite control programs among primary schoolchildren in Lao PDR (2000-2004). The Korean Foundation for International Healthcare (KOFIH), KOICA, and the Ministry of Healthcare of the Lao PDR have backed up a collaborative project between Korea and Lao PDR to control food-borne trematode infections in Laos (2007-2012). This work was supported by a research grant from Chungbuk National University in 2012 for DNA analysis, data processing and statistical analysis. Some parasite materials used in this study were provided by the Parasite Resource Bank of Korea, National Research Center (2012-0000037), Republic of Korea.

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