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Identification of a previously unidentified endemic region for taeniasis in North Sumatra, Indonesia

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ABSTRACT.

In North Sumatra, Indonesia, taeniasis has previously been reported solely from Samosir Island. In 2014, three individuals were identified with taeniasis after voluntarily reporting for treatment, and subsequent investigation conducted in 2017 indicated that a previously unidentified endemic area exists in Simalungun District. Molecular analysis showed the worms to be hybrid-derived descendants of *Taenia asiatica* and *Taenia saginata*, which is consistent with specimens identified previously from Samosir Island.

Keywords: taeniasis; *Taenia asiatica*; North Sumatra; Indonesia

Human taeniasis, caused by *Taenia saginata, Taenia solium* and *Taenia asiatica*, occurs after eating beef, pork or pig viscera contaminated with metacestodes, the larval stage of these parasites. Historically, in Indonesia, *T. asiatica* taeniasis has been exclusively reported in residents of Samosir Island, which is located in Lake Toba, Samosir District, North Sumatra Province (Fig. 1) (Cross et al., 1976; Fan et al., 1988; Kosin et al., 1972, 1988). *T. asiatica* (Eom and Rim, 1993; Fan, 1988; Fan et al., 1988; Hoberg et al., 2000; Ito et al., 2003; Sato et al., 2018) is considered a sister species of *T. saginata* based on molecular phylogeny (Nakao et al., 2013), with the two species being morphologically similar. The life cycles of the parasites do, however, differ with the predominate intermediate host for *T. asiatica* being the pig, in contrast to cattle for *T. saginata*. However, recent molecular analyses have revealed that most *T. asiatica* circulating in Asia, including Indonesia, Thailand, Vietnam, Lao PDR, China and Korea are not pure *T. asiatica*, but hybrid-derived descendants of *T. asiatica* and *T. saginata* (Okamoto et al., 2010; Sato et al., 2018; Yamane et al., 2013). It is believed that pure *T. asiatica* may only now be distributed in Taiwan and the Philippines (Okamoto et al., 2010; Yamane et al. 2013).

The population of North Sumatra consists largely of members of a variety of Batak tribes. Due to a local education program focused on boiling of meat prior to consumption, no *Taenia* tapeworm carriers were confirmed on the island from 2006 onwards (Wandra et al., 2006, 2013). However, in 2014, three taeniasis cases were identified and treated at Dr. Umar Zein Tropical Disease and Infection Clinic in Medan, the capital city of North Sumatra. Two of the carriers lived in Simalungun District, and the third lived in the bordering Serdang Bedagai District (Fig. 1B). These cases indicated the need to further investigate taeniasis in this region. The findings

presented here are the results of a preliminary taeniasis survey conducted in Simalungun District during 2017.

Survey was carried out at the local health center in the village of Nagori Dolok in the Silau Kahaean Sub-District of Simalungun in October and November of 2017. Communications were made to members of 7 villages (population: 10,068) in a province of 13 villages in (population: 16,067) in Silau Kahaean, through health care workers one month prior to commencing fieldwork. Interested and willing volunteers were requested to come forward for testing and susequent treatmenf for taeniasis.

In total, 250 individuals voluntarily participated in the survey, which consisted of a questionnaire used to collect demographic information and history of passing proglottids in the previous six months followed by microscopic stool examination. A total of 180 people were treated with a 600 mg tablet of praziquantel (10 mg/kg for an adult weighing 60 kg) (Biltricide, Bayer, Leverkusen, Germany), followed 1-2 hours later with a 25 mg tablet of Dulcolax laxative (Boehringer Ingelheim, Ingelheim am Rhein, Germany). Treated individuals included those from whom taeniid eggs and proglottids were confirmed (n = 140), those who only expelled proglottids (n = 21), those who only expelled eggs (n = 8), and those without eggs or proglottids, but who requested treatment (n = 11). A total of 171 of 180 people including two of 11 people with no evidence of infection were confirmed to have harbored tapeworms.

Proglottids from all discharged tapeworms were kept in 10% formalin-saline for morphological examination except for one which was fixed in ethanol. There were approximately 16 uterine branches in each gravid proglottid, indicating that the infecting species were all *T. asiatica* (11-32) or *T. saginata* (14-32), but not *T. solium* (5-11) (Fan, 1988). Molecular identification was performed on four specimens (one fixed in ethanol and three fixed in 10% formalin-saline). Genomic DNA was

98 extracted from immature proglottids using the DNeasy Blood and Tissue Kit
99 (QIAGEN, Hilden, Germany) in accordance with the manufacturer's instructions, and
100 then used as a template for polymerase chain reaction (PCR). Both mitochondrial (mt)
101 and nulcear genes were analyzed to evaluate whether the specimens were *T. asiatica*,
102 *T. saginata*, or hybrid-derived descendants of the two species. Partial sequences of the
103 mt *cox*1 gene were amplified using the universal primer set for cestodes (pr-a and pr-
104 b) (Okamoto et al., 1995). The nuclear polymerase delta (*pold*) gene was also
105 sequenced as previously described (Yamane et al., 2013).
106 Partial sequences (361 bp) of the *cox*1 gene were obtained from all four
107 tapeworms. The sequences were identical to each other and the BLAST search
108 revealed that the sequence was the same as numerous previously submitted *T. asiatica*
109 isolates, including an isolate from Samosir Island (AB465228). Therefore, based on
110 mt gene analysis, these specimens are considered *T. asiatica*. Nuclear gene sequences
111 (1,097 bp) were obtained by direct sequencing of PCR product from two worms.
112 Results showed that the *pold*B allele was the same as that found in previously
113 described hybrid-derived descendants and differed from the alleles reported from pure
114 *T. asiatica* (Yamane et al., 2013). This result indicated that these two tapeworms
115 were hybrid-derived descendants of *T. asiatica* and *T. saginata*, which is consistent
116 with specimens identified previously from Samosir Island (Okamoto et al., 2010;
117 Yamane et al., 2013; Wandra et al., 2006).

118 Based on the questionnaire, all confirmed tapeworm carriers (n = 169) were
119 Christians (100%) and the majority of carriers were men (149/169, 88.2%) and palm
120 farmers (141/169, 83.4%). Most confirmed carriers had a history of passing
121 proglottids in the last 6 months (161/169, 95.3%). It is interesting and important to
122 point out that two of additional persons who were not confirmed through the

123 questionnaire and microscopy but requested treatment (n = 11) expelled *Taenia*
124 tapeworms after treatment with PZQ. The youngest carrier was a 12-year-old boy, and
125 the oldest was a 70-year-old man. All carriers (100%) reported eating raw or
126 inadequately cooked pork as part of traditonal dishes. Every event of Wedding party
127 or Cultural party, pieces of undercooked pig liver are served for family leader (men)
128 as honor in Batak culture. Also, almost all customers visiting restaurants where
129 undercooked pig liver is served are men. None of the tapeworm carriers reported
130 eating undercooked beef. Therefore, it is suspected that the infectious source, in the
131 study area, is pork.
132 Although only a preliminary study, the data presented here indicates that there is a
133 taeniasis endemic region in North Sumatra which had previously not been identified.
134 The areas selected for previous studies and interventions, mainly Samosir Island and
135 some lake side of the Lake Toba, were easily accessible and of a higher socio-
136 economic status and it appears that the poorer communicities in the large palm
137 farming areas may have been overlooked when designing programs. Extensive, well
138 designed, studies are now needed to clarify the causative species of taeniasis and to be
139 better understand the epidemiology of the parasite in this locality so that appropriate
140 control programs may be launched (Wandra et al., 2006, 2013).
141 As the prevalence of taeniasis in the 7 villages in Silau Kahaean Sub-District is
142 assumed to be higher than 1.7% (171/10,068). Mass drug administration may be
143 recommended for further studies. However, it is the real serious problem in Indonesia
144 to get PZQ or other drug including niclosamide, highly useful for deworming of
145 tapeworms.
146 In conclusion, we have documented the exisistance of a previoously unidentified
147 endemic foci of taeniasis in the Simalungun District of North Sumatra.

Conflict of interest

The authors declare that there is no conflict of interest.

Author's contributions

UZ conducted the field work with SS, IJ, AHP.

UZ, SS, TWS worked for morphological observation.

MO, TY worked for molecular analysis.

AI coordinated this study when he got data from UZ with UZ, TW and KS.

AI prepared the MS with UZ, TW, MO, TY, KS, TWS, HL.

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References

Cross, J.H., Clarke, M.D., Cole, W.C., Lien, J.C., Partono, F., Joesoef, A., Kosin, E.H. 1976. Parasitology survery in northern Sumatra, Indonesia. J. Trop. Med. Hyg. 79 (6), 123-131.

Eom, K.S., Rim, H.J. 1993. Morphologic description of *Taenia asiatica* sp. n. Korean J. Parasitol. 31 (1), 1-6.

Fan, P.C. 1988. Taiwan *Taenia* and taeniasis. Parasitol. Today 4 (3), 86-88.

Fan, P.C., Chung, W.C., Lin, C.Y., Soh, C.T., Lee, K.T., Kosman, M.L., Kosin, E., Depary, A.A., Napitupulu, T. 1988. Studies on taeniasis in Taiwan VI. Is *Taenia* from Taiwan, Korea and Indonesia a new species? Chinese J. Parasitol. 1, 56-70.

Hoberg, E.P., Jones, A., Rausch, R.L., Eom, K.S., Gardner, S.L. 2000. A phylogeneitc hypothesis for species of the genus *Taenia* (Eucestoda: Taeniidae), J. Parasitol. 86 (1), 89-98.

Ito, A., Nakao, M., Wandra, T. 2003. Human taeniasis and cysticercosis in Asia. Lancet 362 (9399), 1918-1920.

Kosin, E., Depary, A.A., Djohansjah, A. 1972. Taeniasis di Pulau Samosir (Taeniasis on the Samosir Island). Maj. Fak. Kedok., Univ. Sumatra Utara, Medan 3, 5-11. (in Indonesian)

Kosin, E., Kosman, M.L., Fan, P.C., Depari, A.A., Napitupulu, T. 1988. Penelitian taeniasis di pulau Samosir, Sumatra Utara (Study of taeniasis in Samosir Island, North Sumatra). Simp. Parasitol. Klinik 47-53. (in Indonesian)

Nakao, M., Lavikainen, A., Iwaki, T., Haukisalmi, V., Konyaev, S., Oku, Y., Okamoto, M., Ito, A. 2013. Molecular phylogeny of the genus *Taenia* (Cestoda:

Taeniidae): proposas for the resurrection of *Hydatigera* Lamark, 1816 and the creation of a new genus *Versteria*. Int. J. Parasitol. 43 (6), 427-437.

Okamoto, M., Bessho, Y., Kamiya, M., Kurosawa, T., Horii, T. 1995. Phylogenetic relationships within *Taenia taeniaeformis* variants and other taeniid cestodes inferred from the nucleotide sequences of the cytochrome *c* oxidase subunit I gene. Parasitol. Res. 81 (6), 451-458.

Okamoto, M., Nakao, M., Blair, D., Anantaphruti, M.T., Waikagul, J., Ito, A. 2010. Evidence of hybridization between *Taenia saginata* and *Taenia asiatica*. Parasitol. Int. 59 (1), 70-74.

Sato, M.O., Sato, M., Yanagida, T., Waikagul, J., Pongvongsa, T., Sako, Y., Sanguankiat, S., Yoonuan, T., Kounnayang, S., Kawai, S., Ito, A., Okamoto, M., Moji, K. 2018. *Taenia solium*, *Taenia saginata*, *Taenia asiatica*, their hybrids andother helminthic infections occurring in a neglected tropica diseases' highly endemic area in Lao PDR. PLoS Negl. Trop. Dis. 12 (2), e0006260.

Wandra, T., Depary, A.A., Sutisna, P., Margono, S.S., Suroso, T., Okamoto, M, Craig, P.S., Ito, A. 2006. Taeniasis and cysticercosis in Bali and North Sumatra, Indonesia. Parasitol. Int. 55 (Suppl), S155-S160.

Wandra, T., Ito, A., Swastika, K., Dharmawan, N.S., Sako, Y., Okamoto, M. 2013. Taeniases and cysticercosis in Indonesia: past and present situations. Parasitology 140 (13), 1608-1616.

Yamane, K., Yanagida, T., Li, T., Chen, X., Dekumyoy, P., Waikagul, J., Nkouawa, A., Nakao, M., Sako, Y., Ito, A., Sato, H., Okamoto, M. 2013. Genetic relationships between *Taenia saginata*, *Taenia asiatica* and their hybrids. Parasitology 140 (13), 1595-1601.

Fig. 1. Map of Indonesia (A), showing endemic areas of *T. solium* taeniasis and cysticercosis (Wandra et al., 2006, 2013) except North Sumatra where *T. asiatica*, exactly hybrid-derived descendants of *T. asiatica* and *T. saginata*, has been reported from 1970', and Bali where *T. saginata* is rather common than *T. solium* (Wandra et al., 2006, 2013). All other sporadic areas shown in grey, and endemic Papua are exclusively on *T. solium*, and a close-up map of North Sumatra Province (B) showing both Samosir Island where hybrid-derived descendants of *T. asiatica* and *T. saginata* had been recorded, and Simalungun District where they were newly confirmed during 2017.