Development of a Rapid Diagnostic Kit That Uses an Immunochromatographic Device To Detect Antibodies in Human Sparganosis

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A diagnostic kit using an immunochromatographic device was developed to replace the time-consuming immunodiagnostic methods for human sparganosis. The kit was found to be faster and easier to use than an enzyme-linked immunosorbent assay (ELISA) and showed higher sensitivity and specificity. It will be useful for the laboratory diagnosis of hospitalized cases of sparganosis.

Sparganosis is a parasitic zoonosis caused by a larval plerocercoid (sparganum) of tapeworms belonging to the genus Spirometra. As the etiologic agents of the disease, Spirometra erinaceieuropaei (Rudolphi, 1819) Mueller, 1937 and Spirometra mansonioides Mueller, 1935, are important in Asia (1–8) and the Americas (1, 9), respectively.

In the life cycle of the parasite, freshwater copepods are the first intermediate hosts, and frogs, snakes, and chickens are the second intermediate or paratenic hosts. Carnivores, such as dogs and cats, are the definitive hosts. Humans can become infected by consuming raw or undercooked meat from frogs, snakes, or chickens infected with the larval plerocercoid, or by drinking water contaminated with copepods harboring a procercoid, which develops into a plerocercoid (9).

Once a human is infected, the plerocercoid grows and migrates preferentially into subcutaneous tissues and to the abdominal viscera and urogenital organs (1, 5, 6, 9, 10). A potentially painful inflammatory reaction develops in the surrounding tissue, causing the formation of subcutaneous nodules that may appear intermittently (11). The central nervous system and eyes are also affected (5, 11–14).

Although the detection of a plerocercoid is the gold standard method for diagnosing sparganosis, sparganosis is also diagnosed when IgG antibodies specific for plerocercoids are detected in the peripheral blood and cerebrospinal fluid (15–17). Methods for the detection of IgG antibodies have been established, including enzyme-linked immunosorbent assay (ELISA) (15, 18, 19), multidot ELISA (15, 20, 21), immunoblot (22), and two-dimensional immunoblot (23). These methods are time-consuming and require sophisticated equipment. We developed a simple and rapid diagnostic kit (iSpa) using an immunochromatographic device to replace the conventional methods.

To prepare a highly immunogenic cysteine proteinase as a diagnostic antigen (24–26), S. erinaceieuropaei plerocercoids were collected from subcutaneous tissues of a Japanese striped snake (Elaphe quadrivirgata, family Colubridae) and confirmed to be S. erinaceieuropaei by DNA analysis, as reported previously (3). The remaining plerocercoids were treated three times with 10 to 20 volumes of chilled acetone for 20 to 30 min and stored at −20°C. Since the purification method for the cysteine proteinase was only briefly reported previously (25), we describe the protocol in detail here. The defatted plerocercoids (dry weight, 10 g) were ground and then homogenized in 10 mM Tris-HCl (pH 7.6) containing 0.1 M NaCl, 1 mM EDTA, and 1 mM dithiothreitol (DTT) using a Polytron (Kinematica Co., Lucerne, Switzerland). After centrifugation at 170,000 g for 1 h at 4°C, the supernatant was treated with 35 to 50% saturated ammonium sulfate to precipitate the cysteine proteinase-rich protein fraction. After centrifugation at 10,000 g for 1 h, the pellet was resuspended in 5 mM phosphate buffer (pH 7.0) containing 1 mM EDTA and 1 mM DTT and centrifuged at 260,000 g for 1 h. The supernatant was acidified by adding 50 mM acetate buffer (pH 4.0) containing 5 mM EDTA and 1 mM DTT and incubated at 37°C for 90 min. After centrifugation at 10,000 g for 1 h, the supernatant was adjusted to pH 6.0 with NaOH and concentrated in 40 to 65% acetone. After centrifugation at 10,000 g for 1 h, the resultant precipitate was dialyzed against 1 mM NaHCO3 (pH 7.0) containing 1 mM EDTA and 1 mM DTT and then centrifuged at 260,000 g for 1 h. A large amount (80 to ~100 mg) of purified cysteine proteinase was obtained from 10 g of defatted plerocercoids.

The human serum samples examined were as follows: parasitologically confirmed sparganosis cases (n = 13), other parasitic helminthiases (n = 74) that were diagnosed by stool examination and/or serological tests, and healthy volunteers (n = 59) who were proven by parasitological and/or serological tests to be free from any parasitic infections. The use of serum samples was approved by the Medical Ethics Committee of the National Institute of Infectious Diseases, Tokyo, Japan (no. 177), and the Human Ethics

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Committee of Khon Kaen University, based on the Ethics of Human Experimentation of the National Research Council of Thailand (no. HE561396).

The iSpa kit using the cysteine proteinase was optimized based on the ELISA results using the cysteine proteinase at Adtech, Inc., Ltd., Oita, Japan. The ELISA was performed according to a previous report (27), except for the use of the cysteine proteinase (0.5 μg/ml) and peroxidase-labeled protein G (Zymed, South San Francisco, CA, USA). The kit consisted of an immunochromatographic device, sample buffer, and conjugate. The diagnostic reliability of the kit was evaluated as follows: serum samples were diluted 1:5 in sample buffer, and a 5-μl aliquot of the diluted serum was loaded onto the area inscribed "SAMPLE." The cover was opened and one drop of peroxidase-labeled anti-human IgG was spotted onto "DROP" and the cover was closed. Finally, a bag was opened and one drop of peroxidase-labeled anti-human IgG was loaded onto the area inscribed "SAMPLE." The cover case (76.5%) for the iSpa kit was higher than the value (41.9%) in ELISA. The positive predictive value was higher (86.5% due to the increased false-positive cases. These false-positive reactions were likely to be observed when the antibody levels were higher ($\geq 0.8$ OD in ELISA). The positive predictive value ($76.5\%$) for the iSpa kit was higher than the value ($41.9\%$) in ELISA at the prevalence of 8.9% ($13/146$). This indicates that the kit is more applicable for groups with a high prevalence, e.g., hospitalized cases.

Although sparganosis cases in which the antibody levels were monitored over a long period are limited, we tried to monitor antibody levels using two diagnostic tools. Figure 2 shows the change in antibody levels monitored over 2 years after surgical treatment in a cerebral case (28). The antibody level was temporarily elevated after a craniotomy, but the levels decreased 5 months later and then gradually declined. It has been reported that antibody levels decline after 3 to 10 months in sparganosis cases (17, 29–31), and the kinetics of the antibody response after treatment may depend on individual patients and/or tissues affected by plerocercoids. The iSpa kit may be useful for monitoring the time course of recovery.

Sparganosis is a neglected parasitic disease, and there are few public health strategies aimed at the prevention and control of the disease.

### Table 1: Serum samples examined and diagnostic results by iSpa kit and ELISA

| Serum samples from patients with* | iSpa kit | ELISA |
|----------------------------------|----------|-------|
| Sparganosis                      | 13/13    | 13/13 |
| Gnathostomiasis                  | 0/13     | 1/13  |
| Cysticeriosis                    | 1/10     | 5/10  |
| Ascariasis                       | 1/5      | 1/5   |
| Capillaria*                      | 0/5      | 0/5   |
| Anisakiasis                      | 0/2      | 0/2   |
| Spiruroid larva migrans          | 0/1      | 0/1   |
| Strongyloidiasis                 | 0/9      | 2/9   |
| Trichinelliosis                  | 0/2      | 1/2   |
| Dirofilariasis                   | 0/3      | 0/3   |
| Onchocercosis                    | 0/1      | 0/1   |
| Loasis                           | 0/2      | 0/2   |
| Fascioliasis                     | 1/5      | 4/5   |
| Paragonimiasis                   | 1/16     | 3/16  |
| Healthy individuals              | 0/59     | 0/59  |

*Capillaria due to Capillaria philippinensis, spiruroid larva migrans due to Crassicauda giliakiana (36), trichinellosis due to Trichinella papaue (37) and Trichinella spiralis (38), dirofilariasis due to Dirofilaria immitis, onchocercosis due to Mansonella perstans, fascioliastis due to Fasciola gigantica, paragonimiasis due to Paragonimus heterotremus, Paragonimus miyazakii, and Paragonimus westermani.

### Table 2: Comparison of parasitological and immunological diagnostic methods for sparganosis

| Test type and results | No. with plerocercoid presence | Total no. |
|-----------------------|-------------------------------|-----------|
| **iSpa kit**          |                               |           |
| +                     | 13                            | 4         | 17        |
| -                     | 0                             | 129       | 129       |
| **Total**             | 13                            | 133       | 146       |
| **ELISA**             |                               |           |
| +                     | 13                            | 18        | 31        |
| -                     | 0                             | 115       | 115       |
| **Total**             | 13                            | 133       | 146       |
infection; however, immunodiagnostic methods have been well established (15, 18–23). Although the definitive diagnosis of sparganosis is the detection of plerocercoid, there have been reported cases of sparganosis in which plerocercoids were not detected and the diagnosis was based on a decrease in antibody titers and eosinophil counts, as well as an improvement in clinical symptoms; the diagnosis should be considered if an antibody against the diagnostic antigen. A recombinant cysteine proteinase is also available (18). Currently, the iSpa kit has been used as a diagnostic method, but it is a simple and rapid method. The increased sensitivity and specificity of the kit provide a more reliable diagnostic result. It is expected that the iSpa kit will be useful for laboratory diagnosis and for suspected cases for which biopsy and excision procedures are not feasible.

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REFERENCES

1. Bowman DD, Hendrix CM, Lindsay DS, Barr SC. 2002. Feline clinical parasitology. Wiley-Blackwell, NJ.
2. Wiwanitkit V. 2005. A review of human sparganosis in Thailand. Int. J. Infect. Dis. 9:312–316. http://dx.doi.org/10.1016/j.ijid.2004.08.003.
3. Yamasaki H, Nakaya K, Nakao M, Sako Y, Ito A. 2007. Significance of molecular diagnosis using histopathological specimens in cestode zoonoses. Trop. Med. Health 35:307–321. http://dx.doi.org/10.2149 /tmh.35.307.
4. Shin EH, Guk SM, Kim HJ, Lee SH, Choi YJ. 2008. Trends in parasitic diseases in the Republic of Korea. Trends Parasitol. 24:143–150. http://dx. doi.org/10.1016/j.pt.2007.12.003.
5. Yoshikawa M, Ouii Y, Nishiofuku M, Ishizaka S, Nawa Y. 2010. Sparganosis cases reported in Japan in the recent decade, 2000–2009. Clin. Parasitol. 21:33–36. (In Japanese.)
6. Ananthaprutti MT, Nawa Y, Vanvanitcha Y. 2011. Human sparganosis in Thailand: an overview. Acta Trop. 118:171–176. http://dx.doi.org/10 .1016/j.actatropica.2011.03.011.
7. Li MW, Song HQ, Li C, Lin HY, Xie WT, Lin RQ, Zhu XQ. 2011. Sparganosis in mainland China. Int. J. Infect. Dis. 15:e154–e156. http:// dx.doi.org/10.1016/j.ijid.2010.10.001.
8. Yamasaki H. 2013. Food-borne parasitic infection (8) Helminthic infection (3): Cestodes. J. Antibact. Antifung. Agents. 41:227–236. (In Japanese.)
9. Mueller JF. 1974. The biology of Spirometra. J. Parasitol. 60:3–14.
10. Holodniy M, Almenoff J, Loutit J, Steinberg GK. 1991. Cerebral sparganosis: case report and review. Rev. Infect. Dis. 13:155–159. http://dx.doi .org/10.1093/clinids/12.5.155.
11. Otranto D, Eberhard ML. 2011. Zoonotic helminths affecting the human eyes. Parasit. Vectors 4:41. http://dx.doi.org/10.1186/1756 -3305-4-41.
12. Song T, Wang WS, Zhou BR, Mai WW, Li ZZ, Guo HC, Zhou F. 2007. CT and MR characteristics of cerebral sparganosis. AJNR Am. J. Neuroradiol. 28:1700–1705. http://dx.doi.org/10.3174/ajnr.A0659.
13. Lv S, Zhang Y, Steinmann P, Zhou XN, Utzinger J. 2010. Helminth infections of the central nervous system occurring in Southeast Asia and the Far East. Adv. Parasitol. 72:376–381. http://dx.doi.org/10.1016/S0065 -308X(10)72012-1.
14. Sawai K, Yamasaki H, Ito A, Miyajima H. 2010. Cerebral sparganosis: the wandering lesion. Neurology 74:180. http://dx.doi.org/10.1212 /WNL.0b013e3181c91a15.
15. Araki K. 2003. Immunodiagnosis of parasitic diseases. JIM 13:254–256. (In Japanese.)
16. Taguchi S, Ishii Y, Umebayashi Y, Ohmae H. 2005. Sparganosis. Pract. Dermatol. 27:571–575. (In Japanese.)
17. Yamasaki S, Horio T, Uemura Y, Ishida T, Nishiyama T. 2007. A case of sparganosis mansoni. Hifu no kakaga 6:38–43. (In Japanese with English summary.)
18. Nishiyama T, Ide T, Himes SR, Jr, Ishizaka S, Araki T. 1994. Immunodiagnosis of human sparganosis mansoni by micro-chemiluminescence enzyme-linked immunosorbent assay. Trans. R. Soc. Trop. Med. Hyg. 88:663–665. http://dx.doi.org/10.1016/S0035-9203(94)90218-6.
19. Cui J, Li N, Wang ZQ, Jiang P, Lin XM. 2011. Serodiagnosis of experimental sparganum infections of mice and human sparganosis by ELISA using ES antigens of Spirometra mansoni. Parasitol. Res. 108:1551–1556. http://dx.doi.org/10.1007/s00436-010-2206-2.
20. Nakamura-Uchiyama F. 2005. Multi dot-ELISA test for parasitic diseases. Clin. Parasitol. 16:23–25. (In Japanese.)
21. Nakasone N, Uehara E, Korenaga M, Yamaguchi S, Takahashi K, Uezato H. 2010. A case of sparganosis mansoni manifesting as creeping eruption. Nishinihon J. Dermatol. 74:31–36. (In Japanese with English summary.)
22. Song CY, Choi DH, Kim TS, Lee SH. 1992. Isolation and partial char-acterization of cysteine proteinase from sparganum. Kisaengchunghak Chaphchi. 30:191–199.
23. Rahman M, Lee EG, Bae YA. 2011. Two-dimensional immunoblot anal-ysis of antigenic proteins of Spirometra plerocercoid recognized by human
patient sera. Parasitol. Int. 60:139–143. http://dx.doi.org/10.1016/j.parint.2011.01.002.
24. Song CY, Chappell CL. 1993. Purification and partial characterization of cysteine proteinase from Spirometra mansoni plerocercoids. J. Parasitol. 79:517–524. http://dx.doi.org/10.1093/PHLS/3595, http://dx.doi.org/10.2307/3283376.
25. Nakamura T, Take-Watanabe A, Yanagisawa T. 1996. Cleavage of muscle structural proteins with cysteiny protease from Spirometra erinaceieuropaei plerocercoid. Jpn. J. Parasitol. 45:185–191.
26. Kong Y, Kang SY, Kim SH, Chung YB, Cho SY. 1997. A neutral cysteine protease of Spirometra mansoni plerocercoid invoking an IgE response. Parasitology 114:263–271. http://dx.doi.org/10.1017/S0031182096008529.
27. Yamasaki H, Araki K, Lim PK, Zasmy N, Mak JW, Taib R, Aoki T. 2000. Development of a highly specific recombinant Toxocara canis second-stage larva excretory-secretory antigen for immunodiagnosis of human toxocariasis. J. Clin. Microbiol. 38:1409–1413.
28. Masuya M, Aizawa H, Shirai W, Tokumitsu N, Sako K, Sugano S. 2008. A case of sparganosis resected alive plerocercoid. J. Noryo City Hosp. 16:33–36. (In Japanese.)
29. Watanabe M, Kunii T, Tanoue H, Sugaya H, Matsuda S, Ohteki T. 2004. A case of sparganosis possibly linked to horse meat consumption. Clin. Dermatol. 58:543–546. (In Japanese.)
30. Ono M, Furuba S, Hisatomi M, Kitayama A, Misuna N, Narusawa H. 2011. A case of sparganosis. Nishinomi J. Dermatol. 73:241–244. (In Japanese.)
31. Horiguchi Y, Yamada M. 2013. A case of sparganosis mansoni caused by intake of raw eggs of soft-shell turtle. Hifu no kagaku 12:39–43. (In Japanese with English summary.)
32. Yamasaki H, Araki K, Aoki T. 1994. Parasitic diseases confirmed in Juntendo University. Juntendo Med. J. 40:262–279. (In Japanese.)
33. Tanaka S, Maruyama H, Ishikawa K, Nawa Y. 1997. A case report of pleural sparganosis. Parasitol. Int. 46:73–75. http://dx.doi.org/10.1016/S1383-5769(97)00001-9.
34. Liu DW, Kato H, Nakamura T, Sugane K. 1996. Molecular cloning and expression of the gene encoding a cysteine proteinase of Spirometra erinacei. Mol. Biochem. Parasitol. 76:11–21. http://dx.doi.org/10.1016/0166-6851(95)02522-7.
35. Rahman SM, Kim JH, Hong ST, Choi MH. 2014. Diagnostic efficacy of a recombinant cysteine protease of Spirometra erinacei larvae for serodiagnosis of sparganosis. Korean J. Parasitol. 52:41–46. http://dx.doi.org/10.3347/kjp.2014.52.1.41.
36. Mizuno M, Shimizu Y, Sakai H, Shirabe YH, Sugiyama H, Yamasaki H. 2013. A case of spirurina larva creeping disease initially treated as subileus. Clin. Dermatol. 67:539–542. (In Japanese.)
37. Intapan PM, Chotmongkol V, Tantrawatpan C, Sanpool O, Morakote N, Maleewong W. 2011. Molecular identification of Trichinella papuae from a Thai patient with imported trichinellosis. Am. J. Trop. Med. Hyg. 84:994–997. http://dx.doi.org/10.4269/ajtmh.2011.10-0675.
38. Morakote N, Sukhavat K, Khamboonruang C, Siriprasert V, Suphawitayanukul S, Thamasonthi W. 1992. Persistence of IgG, IgM, and IgE antibodies in human trichinosis. Trop. Med. Parasitol. 43:167–169.