Parasitology International 70 (2019) 1–4

Contents lists available at ScienceDirect

Parasitology International

journal homepage: www.elsevier.com/locate/parint

Short Communication

Ascaris lumbricoides found in ashore corpses from Korean peninsula to Japan

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ARTICLE INFO

Keywords:
Ascaris lumbricoides
North Korea
Imported infectious diseases
Drifting ashore
Roundworm
Forced immigration

ABSTRACT

Yearly, several reports of unknown boats and corpses brought by the Tsushima Current are found ashore Japanese coast. Niigata prefecture had the highest number of the drifting ashore corpses in Japan with 45.7% (16/35) in 2017. Corpses from North Korea, confirmed by documents and photos were autopsied and in 3/16 was possible to recover worms full of eggs, morphologically identified as ascarids. Further molecular analysis of ITS1, 5.8S rDNA and ITS2 sequences confirmed all specimens were *Ascaris lumbricoides*. The contamination level by *Ascaris lumbricoides* eggs in the coast, the health impact and consequences of the epidemiological bridging produced by this forced migration in public health should be investigated. Moreover, control of helminthiases might be a necessary task in North Korea.

1. Introduction

The Sea of Japan is bounded by Japan and Sakhalin Island to the east and by Russia and Korea on the Asian mainland to the west. With the Liman Cold Current from the Sea of Okhotsk in the North and the Tsushima Warm Current flowing into the Japan Sea from the East China Sea the debris are usually brought to Japan coast [1]. Yearly, several unknown boats and corpses are found ashore Japanese coast with several reports of boats and corpses suspected to come from Korean peninsula [2]. They are identified by North Korean flags, documents and photos as direct proofs of North Korean origin scattered around the findings (ships, clothes, utensils, and bodies). The number of the drifting ashore cases greatly increased from November of 2017 ($N = 28$), and in December solely it reached to 45 cases, reaching a total of 104 cases in the year (Fig. 1). In 2017, 42 survivors were encountered, 35 corpses and uncountable skeletonized bodies were found according to Japan Costal Guard report (Table 1).

Regarding the epidemiology of infectious disease, the association between the introduction of pathogens and migration is a known fact. A recent example is the dengue fever brought to Japan from travelers [3], trypanosomiasis cases in non-endemic countries and several others [4]. Migration dynamics and infectious diseases burden are related, as described in Mediterranean Sea [5]. Border health and quarantine medicine practices were established attempting to control the importation of diseases [4]. Respiratory infections, gastrointestinal and diarrheal diseases, are issues to concern due the transmission pattern and once the diseases are introduced from one endemic regions to a non/very low endemicity area can cause high rates of death and morbidity due to the number of susceptible individuals [6].

The immigrant origin region may reflect the potential risks of infectious disease acquisition/transmission [4]. A recent report showed malaria and parasitic diseases as major class of infectious diseases in the country North Korea [7]. Previous studies showed helminths are frequent infections in North Korea with high prevalence of ascariasis and trichuriasis [8,9].

There is an increasing number of corpses found in Japan coast, but the potential effect of this phenomena in public health is unknown, if some pathogens could be spread by such uncommon migration. We examined the cases found in Niigata prefecture, Japan and identified *Ascaris lumbricoides* from autopsied bodies found ashore.

2. Material and methods

2.1. Parasites

In Niigata Prefecture, from January to December 2017, 16 corpses found ashore with North Korea origin were autopsied. Parasite material were collected from 3 bodies presenting recognizable nematodes. After morphological analysis the material was frozen at -80 °C for further procedures.

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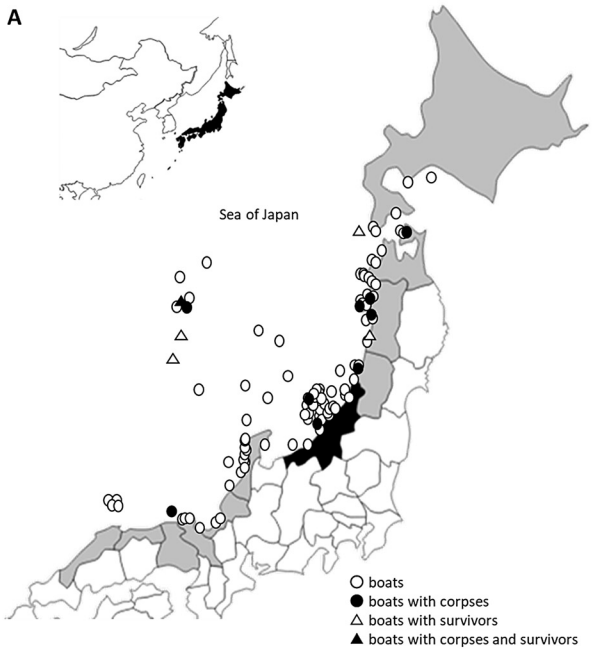
https://doi.org/10.1016/j.parint.2019.01.002
Received 30 November 2018; Received in revised form 21 December 2018; Accepted 6 January 2019
Available online 07 January 2019
1383-5769/



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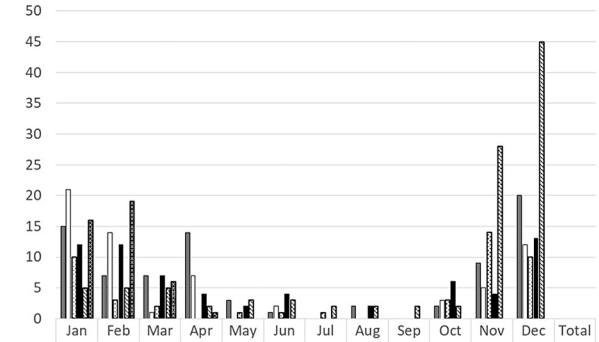


	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Total
2013	15	7	7	14	3	1	0	2	0	2	9	20	80
2014	21	14	1	7	0	2	0	0	0	3	5	12	65
2015	10	3	2	0	1	1	1	0	0	3	14	10	45
2016	12	12	7	4	2	4	0	2	0	6	4	13	66
2017	5	5	5	2	3	3	2	2	2	2	28	45	104
2018	16	19	6	1									42

Fig. 1. A. The prefectures in where the drifting wooden boats/corpse body arrived (Gray areas) in 2017 to April 2018, and the location of Niigata Prefecture (Black). (modified from Japan Costal Guard report, 19th of April 2018), B. The numbers and transition of annual/monthly the drifting wooden boats/corpse body found in Japan in 2013 to 2018, April.

Table 1
The numbers of found corpses/survivors in 2013 to 2018, April in Japan.

Year	Total	No. of the drifting boats with corpses (corpses no.)	No. of the drifting boats with survivors (survivors no.)
2013	80	2 (4)	0
2014	60	0	1 (4)
2015	45	8 (27)	1 (1)
2016	66	2 (11)	0
2017	104	10 (35)	5 (42)
2018[a]	42	2 (9)	0

[a] For 2018, the number is counted until 19th of April.

2.2. DNA purification, sequencing and molecular analysis

Genomic DNA of the parasites was purified from 300 mg of each sample. The material was disrupted with a μT-12 beads crusher (TAITEC Co., Koshigaya, Japan) using 3 stainless steel beads of 4 mm plus 200 mg of 0.2 mm glass beads in each tube. DNA was extracted from the homogenized solution using the High Pure PCR template Preparation Kit (Roche, Hilden, Germany) following the manufacturer's instructions. Final DNA elution was done in 200 μl of elution buffer.

PCR and sequencing targeting first internal transcribed space (ITS1), 5.8S rDNA and internal transcribed spacer 2 (ITS2) was carried out as described by Zhu et al. [10]. Briefly: Using a T100 Thermal Cycler DNA thermocycler (BIO-RAD, Hercules, CA, USA) PCR was performed with KOD FX Neo® Master Mix (TOYOBO, Osaka, Japan) using the primers NC5 (5′-GTAGGTGAACCTGCGGAAGGATCATT-3′) and NC2 (5′-TTAG TTTCTTTTCCTCCGCT-3′) for amplification. The PCR products were sequenced by ABI3730XL sequencer (Applied Biosystems, USA) at Dokkyo Medical University (Japan) with the primers NC5, XZ5R (5′-CGCCGACTGCTATTACATCA-3′), NC13 (5′-ATCGATGAAGAACGC AGC-3′), NC13R (5′-GCTGCGTTCTTCATCGAT-3′), XZ1R (5′-GGAATG AACCCGATGGCGCAAT-3′) and NC2. The obtained sequences were aligned and compared with the same gene sequences from GenBank database using BioEdit version 7.1.3 [11]. A phylogenetic tree was reconstructed by using the neighbor-joining method with MEGA version 6.0 [12]. The tree was evaluated by a bootstrap test based on 1000 resamplings.

3. Results and discussion

During 4 years from 2013 to 2016, 256 wood made boats have been found/arrived at Sea of Japan costal area according to Japan Costal Guard report (Fig. 1) in 12 prefectures of Japan coastal area including Niigata prefecture. With 240 km of coast, Niigata is the prefecture with the longest coast along the Sea of Japan with Sado Island.

In Niigata Prefecture, 36 wooden boats and 16 corpses had arrived in 2017 according to Niigata Prefectural police report. Therefore, from a total of 35 corpses reported in Japan, Niigata prefecture showed the highest number in the country with 45.7% (16/35) of cases. The autopsies revealed 3 of 16 corpses found in Niigata prefecture presenting intestinal parasites, with 3 worms collected from each corps. The obtained worms were damaged due to the stage of decomposition of the host body, however the measures were done, and the sizes varied from approximately 9 to 13 cm length, 5 mm width (Fig. 2). They were morphologically identified to be *Ascaris* spp. by their size and characteristics of the anterior extremity having elongated cylindrical shape with the mouth surrounded by 3 well-developed lips. However, due to similar morphological characteristics of *Ascaris suum*; the pig round worm and *A. lumbricoides*, [13], and the possibility of cross-transmission involving these 2 species of nematode with *A. lumbricoides* infecting pigs and *A. suum* infecting humans [14] further investigation was necessary to identify the found *Ascaris* spp. worms.

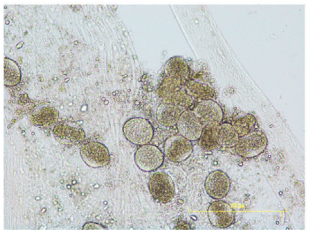
We successfully purified genomic DNA of the 3 samples obtained and proceeded the molecular identification inferred by the first internal transcribed space (ITS1), 5.8S rDNA and internal transcribed spacer 2 (ITS2) region sequences (Fig. 2). *A. lumbricoides* and *A. suum* have quite similar nucleotide sequences, and there is a debate if it should be considered as a single species [15,16]. However, there are six (1.3%) nucleotides in ITS1 region differentiating the two species [10]. The alignment and analysis of the obtained sequences revealed three of the nematodes found in Niigata were *A. lumbricoides* (Fig. 2C).

The direct or indirect exposure to pigs can be a factor facilitating the occurrence of *A. suum* in humans [17], with pigs as the main source of human *Ascaris* infections in areas with no or low *Ascaris* prevalence

A

Parasite		Host (Corpses)		
Sample No.	Length (cm)	Age (y.o.)	Sex	Height (cm)
1	11	About 20-40	M	168
2	9	About 50-60	M	173
3	13	About 40	M	174

B



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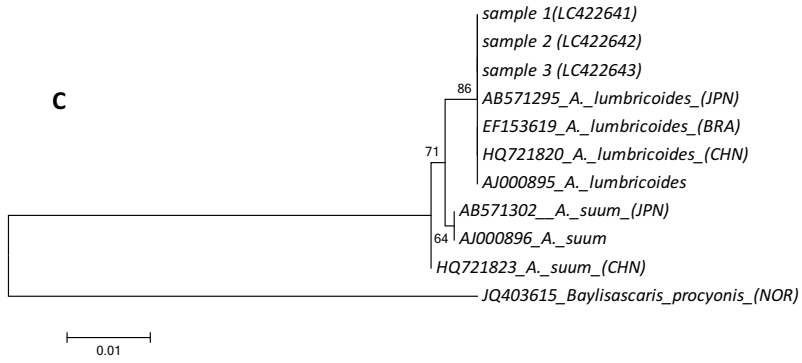

Fig. 2. A. The three nematodes recovered from the corpses and the information about the host, B. Eggs obtained from sample 3, C. Phylogenetic tree constructed by the neighbor-joining method and based on the ITS1, 5.8S rDNA and ITS2 region sequencing of the adult nematodes recovered from the corpses (sample 1–3). Sequence was deposited in GenBank (LC422641, LC422642 and IC422643). Values at nodes represent a bootstrap confidence level > 60% based on 1000 re-samplings.

[18]. In other hand, the high environmental contamination, sometimes by the use of night soil as fertilizer, in endemic areas facilitate and perpetuates the occurrence of *A. lumbricoides* in humans [19]. Additionally, the health status and hygiene can influence the occurrence of *A. lumbricoides*, with higher prevalence in children and almost zero in immunocompetent adults [19].

Malaria and parasitic diseases are a major class of infectious diseases in North Korea [7]. Previous studies showed helminths are frequent infections in North Korea. In 2008, Hong's team presented the results of parasitic examination of 236 residents living in North Hamgyeong Province, near the border between the countries, and it was presented that ascariasis and *Trichuris trichiura* infection rates were 43.2 and 40.3%, respectively [9]. In a 2006 investigation by the Korea Centers for Disease Control and Prevention reported that 434/1501 (28.9%) North Korean defectors were infected with intestinal parasites [8]. For bacterial infection, it is noted that North Korea has been paying attention from World Health Organization (WHO), since North Korea is one of the 30 nations with a high burden of TB and multidrug-resistant TB [7].

To find adults parasitized by *A. lumbricoides* provided information on the health status of the workers in the North Korea, the possible human-human and pig-human transmission points to an existing high environmental contamination as showed by Hall et al. [20] with rapid reinfection after 3 treatments in Bangladesh, a high endemic area. Another potential point of concern is the environmental contamination with eggs of *A. lumbricoides* in the beaches where the corpses are found, once it is known the high resistance of ascaris eggs [21] and its longevity of several years in soil [22] allied to the predisposition of the sea currents to bring materials to Japanese coast [1]. The level of environmental contamination of *Ascaris* eggs in coastal area of Japan is unknown and needs to be studied to assess the risk of infection. People living or visiting those areas and especially children, can eventually be infected by this soil transmitted helminth [19]. In highly endemic countries, periodic mass treatment of helminth infections is simple and cost-effective, especially when targeted at groups such as school-children [23]. On other hand, in areas with medium to high transmission, an expansion of mass drug administration (MDA) targeting beyond school-age children to include preschool and adults is recommended to achieve elimination/control *A. lumbricoides* [24]. In Japan there is no ongoing systematic control for geohelminths.

The health impact and consequences of the epidemiological bridging produced by migration is directly related to the difference of prevalence between origin and the destination and the size of the population that moving between the different disease prevalence patterns [4].

This study shows Japan, as the main destination of drifting materials in Japan Sea because of the ocean currents, should care those ashore arriving cases as an important health issue of imported infectious diseases, as *Ascaris lumbricoides* found in this study, not only social event. Moreover, health should not have borders, measures for control of helminthiases might be urgent and necessary tasks in North Korea.

References

[1] T. Miita, K. Kawatate, Trajectories of drift bottles released in the Tsushima Strait, Prog. Oceanogr. 17 (1986) 255–263.
[2] Japan Coast Guard (JCG), Japan Coast Guard Annual Report 2017, Available at, 2018. https://www.kaiho.mlit.go.jp.
[3] M. Tsuboi, S. Kutsuna, T. Maeki, S. Taniguchi, S. Tajima, F. Kato, C.-K. Lim, M. Saijo, S. Takaya, Y. Katanami, Y. Kato, N. Ohmagari, Dengue virus type 2 in travelers returning to Japan from Sri Lanka, 2017, Emerg. Infect. Dis. 23 (2017) 1931–1933, https://doi.org/10.3201/eid2311.171293.
[4] B.D. Gushulak, D.W. MacPherson, Globalization of infectious diseases: the impact of migration, Clin. Inf. Dis. 38 (2004) 1742–1748, https://doi.org/10.1086/421268.
[5] C. Napoli, M.G. Dente, T. Kärki, F. Riccardo, P. Rossi, S. Declich, Network for the control of cross-border health threats in the Mediterranean basin and black sea, screening for infectious diseases among newly arrived migrants: experiences and practices in non-EU countries of the Mediterranean basin and black sea, Int. J. Environ. Res. Public Health 12 (2015) 15550–15558, https://doi.org/10.3390/ijerph121215002.
[6] World Health Organization (WHO), The Evolution of Diarrhoeal and Acute Respiratory Disease Control at WHO: Achievements 1980–1995 in Research, Development and Implementation. WHO/CHS/CAH/99.12, 1999, www.who.int/child-adolescent-health/New_Publications/CHILD_HEALTH/WHO_CHS_CAH_99.12.pdf.
[7] D.H. Park, M.H. Choi, A.Y. Lim, H.Y. Shin, An analysis of infectious disease research trends in medical journals from North Korea, J. Prev. Med. Pub. Health 51 (2018)

109–120.
[8] S.H. Kim, Strategies for the Development of Health System in North Korea through Sustainable Cooperation and Development, Ministry of Health and Welfare, Seoul, 2008, p. 233 (Korean).
[9] S.T. Hong, H.W. Yoon, M.H. Choi, S.O. Lee, S.H. Shin, Situation of Parasitic Infections in North Korea, Seoul National University, Seoul, 2008 20pp, Korean.
[10] X. Zhu, N.B. Chilton, D.E. Jacobs, J. Boes, R.B. Gasser, Characterisation of *Ascaris* from human and pig hosts by nuclear ribosomal DNA sequences, Int. J. Parasitol. 29 (1999) 469–478.
[11] T.A. Hall, BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT, Nucl. ACS Symp. Ser. 41 (1999) 95–98.
[12] K. Tamura, G. Stecher, D. Peterson, A. Filipski, S. Kumar, MEGA6: molecular evolutionary genetics analysis version 6.0, Mol. Biol. Evol. 30 (2013) 2725–2729.
[13] J.F.A. Sprent, Anatomical distinction between human and pig strains of *Ascaris*, Nature 170 (1952) 627–628.
[14] I. Takata, Experimental infection of man with *Ascaris* of man and the pig, Kitasato Arch. Exp. Med. 23 (1951) 151.
[15] G.H. Liu, C.Y. Wu, H.Q. Song, S.J. Wei, M.J. Xu, R.Q. Lin, G.H. Zhao, S.Y. Huang, X.Q. Zhu, Comparative analyses of the complete mitochondrial genomes of *Ascaris lumbricoides* and *Ascaris suum* from humans and pigs, Gene 492 (2012) 110–116.
[16] D. Leles, S.L. Gardner, K. Reinhard, A. Iñiguez, A. Araujo, Are *Ascaris lumbricoides* and *Ascaris suum* a single species? Parasit. Vectors 5 (2012) 42.
[17] L.A. Miller, K. Colby, S.E. Manning, D. Hoenig, E. McEvoy, E. Montgomery, et al., Ascariasis in humans and pigs on small-scale farms, Maine, USA, 2010-2013, Emerg. Inf. Dis. 21 (2015) 332–334.
[18] R.P. Bendall, M. Barlow, M. Betson, J. Stothard, P. Nejsum, Zoonotic ascariasis, United Kingdom, Emerg. Inf. Dis. 17 (2011) 1964–1966.
[19] S.A. Schüle, P. Clowes, I. Kroidl, D.O. Kowuor, A. Nsojo, C. Mangu, H. Riess, C. Geldmacher, R.P. Laubender, S. Mhina, L. Maboko, T. Loscher, M. Hoelscher, E. Saathoff, *Ascaris lumbricoides* infection and its relation to environmental factors in the Mbeya Region of Tanzania, a cross-sectional, population-based study, PLoS One 9 (2014) e92032, , https://doi.org/10.1371/journal.pone.0092032.
[20] A. Hall, K.S. Anwar, A.M. Tomkins, Intensity of reinfection with *Ascaris lumbricoides* and its implications for parasite control, Lancet 23 (1992) 1253–1257.
[21] S. Yoshida, On the development of *Ascaris lumbricoides* L, J. Parasitol. 5 (1919) 105–115.
[22] R.G. Feachem, D.J. Bradley, H. Garelick, D.D. Mara, *Ascaris* and ascariasis. Chapter 23, in: R.G. Feachem, D.J. Bradley, H. Garelick, D.D. Mara (Eds.), Sanitation and Disease: Health Aspects of Excreta and Waste Water Management. World Bank Studies in Water Supply and Sanitation World Bank, Wiley, Chichester, UK, 1983, pp. 375–399.
[23] D.A.P. Bundy, L.J. Appleby, M. Bradley, K. Croke, T.D. Hollingsworth, R. Pullan, H.C. Turner, N. de Silva, Chapter four - 100 years of mass deworming programmes: a policy perspective from the world bank's disease control priorities analyses, Adv. Parasitol. 100 (2018) 127–154, https://doi.org/10.1016/bs.apar.2018.03.005.
[24] J.E. Truscott, T.D. Hollingsworth, S.J. Brooker, R.M. Anderson, Can chemotherapy alone eliminate the transmission of soil transmitted helminths? Parasit. Vectors 7 (2014) 266, https://doi.org/10.1186/1756-3305-7-266.