

Epidemiology of Domestically Acquired Amebiasis in Japan, 2000–2013

Masahiro Ishikane, Yuzo Arima,* Atsuhiko Kanayama, Takuri Takahashi, Takuya Yamagishi, Yuichiro Yahata, Tamano Matsui, Tomimasa Sunagawa, Tomoyoshi Nozaki, and Kazunori Oishi

Field Epidemiology Training Program, National Institute of Infectious Diseases, Tokyo, Japan; Division of Global Infectious Diseases, Department of Infection and Epidemiology, Graduate School of Medicine, Tohoku University, Miyagi, Japan; Infectious Disease Surveillance Center, National Institute of Infectious Diseases, Tokyo, Japan; Department of Global Infectious Diseases and Tropical Medicine, National Defense Medical College, Saitama, Japan; Department of Parasitology, National Institute of Infectious Diseases, Tokyo, Japan

Abstract. Notifications of amebiasis have been increasing in Japan. Using national surveillance data during 2000–2013, reported cases of amebiasis were analyzed. A case of amebiasis was defined as laboratory-confirmed *Entamoeba histolytica* infection, regardless of presence of symptoms. We described temporal trends and analyzed correlates of asymptomatic versus symptomatic cases based on odds ratios (ORs) and 95% confidence intervals (CIs) using logistic regression. Of 9,946 cases reported during 2000–2013, 7,403 were domestic cases. During this period, the proportion of domestic cases increased from 63% to 85%. Among male cases, majority were middle aged, and from 2008, the number of cases attributed to heterosexual contact surpassed that of homosexual contact. During 2010–2013, increase in notifications was associated with asymptomatic cases, colonoscopy diagnosis, and males with unknown or heterosexual route of infection. Among males, colonoscopy (OR = 31.5; 95% CI = 14.0–71.0) and cases with unknown route of infection, relative to homosexual contact (OR = 2.2; 95% CI = 1.3–3.9), were associated with asymptomatic infections in multivariate analysis. Although the recent rise may have been due to enhanced detection by colonoscopy or reporting, the large number of asymptomatic cases, with reportedly unknown or heterosexual route of infection, has led to a better understanding of amebiasis in Japan and highlights the potential public health concern.

INTRODUCTION

Amebiasis is caused by *Entamoeba histolytica* infection, and invasive amebiasis is the fourth leading cause of death and the third leading cause of morbidity due to protozoan infections worldwide, resulting in ~70,000 deaths each year.^{1,2} Most amebiasis are asymptomatic or cause mild disease. However, 4–10% of patients develop invasive amebiasis that includes extraintestinal infection, which may result in severe and potentially fatal illness.^{1,3–6} Also, substantial health-care costs are necessary to care for invasive amebiasis.⁷ Recent studies have reported that invasive amebiasis is prevalent not only in developing countries due to contaminated food or water, but also in developed areas of east Asia (e.g., China, Japan, Korea, and Taiwan) and Australia as a sexually transmitted infection via oral–anal sexual contact, particularly among men who have sex with men (MSM).^{8–10}

Amebiasis is a nationally notifiable disease in Japan, which is one of the few places, along with Taiwan, to conduct national surveillance for amebiasis.¹¹ Physicians are required to report all cases of amebiasis to the National Epidemiological Surveillance of Infectious Diseases (NESID) system in Japan within 7 days of case diagnosis. Until recently, most reports of amebiasis in Japan were associated with travel to areas with poor hygiene and sanitation. However, the notification rate of domestically acquired amebiasis has been increasing according to data from NESID.^{12,13} And, for domestically acquired infections, while MSM have been known to be a high risk group,^{14–16} recently, there have been clinical reports of non-MSM, particularly mild cases, diagnosed by colonoscopy in Japan.^{17–19}

Therefore, to assess the possible reasons for the recent increase in domestically acquired infections, our objective in this study was to describe the recent epidemiology of

domestic amebiasis in Japan, with a particular focus on potential risk groups.

MATERIALS AND METHODS

Study design. This is a retrospective, exploratory, mostly descriptive epidemiologic study, based on national surveillance data and national population statistics. We assessed the temporal trend and demographic characteristics of notifications of domestic cases of amebiasis between 2000 and 2013, particularly focusing on the period between 2010 and 2013 when a large increase was observed. Furthermore, we compared the characteristics of asymptomatic and symptomatic cases during 2010–2013.

Data sources. In compliance with the Act on Prevention of Infectious Diseases and Medical Care for Patients Suffering Infectious Diseases (the Infectious Diseases Control Law) enacted in April 1999, amebiasis was classified as a category IV notifiable infectious disease, which was then changed to a category V notifiable infectious disease by the partial amendment of the Infectious Diseases Control Law in November 2003. Since April 2006, notification of amebiasis requires clarification of the disease type, that is, intestinal and/or extraintestinal amebiasis, and since February 2011, clinicians have been able to select “colon lesion” (detected primarily by colonoscopy) from a list of clinical manifestations (previously recorded as free text by clinicians).

Notification rates were calculated using national population statistics from Population Survey Reports of the Ministry of Health, Labour and Welfare, Japan, between 2000 and 2013, where the population was estimated as of October 1 each year.²⁰

Criteria for case reporting. Diagnosis can be made by any of the following four detection methods in stool, colonic mucosa, or abscess fluid samples: detection of 1) trophozoites or cysts by visual detection (microscopy), 2) specific antigen by enzyme-linked immunosorbent assay (ELISA), 3) *E. histolytica* DNA by real-time polymerase chain reaction (PCR), and

* Address correspondence to Yuzo Arima, Infectious Disease Surveillance Center, National Institute of Infectious Diseases, 1-23-1 Toyama, Shinjuku-ku, Tokyo 162-8640, Japan. E-mail: arima@niid.go.jp

4) serum antibodies. Standardized methods for laboratory tests are provided to local public health centers under the guidance of the National Institute of Infectious Diseases (NIID). For ELISA and serology, specific positive and negative controls are recommended to local public health centers under the guidance of NIID. For PCR, this guidance recommends the single round PCR assay for which primers were designed based on the reported *E. histolytica* and *Entamoeba dispar* small-subunit rRNA gene sequences, and have been shown to specifically differentiate each other.²¹ Confirmation by any of the above four methods are considered to be laboratory-confirmed *E. histolytica* infection in Japan,^{22,23} and such cases reported to NESID from 2000 to 2013 were included in this study.

The following data regarding cases were extracted from the NESID database: demographics, travel history, suspected route of infection, diagnosis method, clinical manifestations, and disease type (intestinal and/or extraintestinal). Considering the age at which colonoscopy becomes common for health screening purposes, we categorized age dichotomously at 50 years, as previously analyzed.¹⁵ Cases were defined as domestic or imported based on the judgement of the physicians who treated the cases, according to the patients' reported travel history. For the suspected route of infection, physicians could choose from the following five routes: 1) homosexual (sexual contact with someone of the same gender), 2) heterosexual (sexual contact with someone of the opposite gender), 3) sexual unspecified (indicated as sexual infection but not specified as homosexual or heterosexual), 4) nonsexual (infection attributed to contaminated food or water), and 5) unknown (none of (1) through (4) apply). In this study, cases with specific colonic mucosa lesions, such as erythema, edematous mucosa, erosion, white exudates, and ulcers,^{15,17} but otherwise asymptomatic and reported through NESID, were classified as asymptomatic cases.

Statistical analysis. The number and rate of notifications and the distribution of characteristics from 2010 to 2013 were described. Using logistic regression, univariate analysis using odds ratio (OR) and 95% confidence intervals (CIs) was used to compare characteristics between asymptomatic and

symptomatic cases, stratified by sex. Variables with a *P* value of < 0.2 in univariate analysis or hypothesized to be a priori clinically or epidemiology important were included in a multivariate model.

Statistical significance was defined at two-sided *P* values of < 0.05. All statistical analyses were performed with SPSS version 18 (SPSS Inc., Chicago, IL).

Ethics. No ethical approval was necessary because this study was conducted for public health purposes using national surveillance data.

RESULTS

Description of domestic cases between 2000 and 2013. A total of 9,946 cases of amebiasis, including 7,403 domestic cases, were reported during 2000–2013. The annual notification rate rose steadily from 0.30/100,000 in 2000 (*N* = 381) to 0.82/100,000 in 2013 (*N* = 1,047) (Figure 1). The methods used most commonly for confirmation were detection of trophozoites or cysts (7,971/9,946 [80.1%]) and serum antibodies (2,728/9,946 [27.4%]) (includes cases confirmed by more than one diagnosis method).

During 2000–2013, the absolute number and proportion of domestic cases increased considerably from 246 (64.5%) to 889 (84.9%). For domestic cases reported during 2000–2013, the majority were male, ranging from 85.3% in 2007 (*N* = 452) to 90.6% in 2003 (*N* = 319). During 2000–2013, among males, the proportion of cases recorded as unknown route of infection (ranging from 44.3% to 58.7%) was consistently higher than those recorded as sexually acquired (ranging from 29.5% to 41.9%). For females, the proportion of cases recorded as unknown route of infection ranged from 34.6% to 57.7% and sexually acquired cases ranged from 23.1% to 44.0%.

When further stratified by type of sexual contact among males, the number of cases among heterosexual males increased at a greater rate compared with homosexual males, and from 2008, the number of male cases acquired from heterosexual contact surpassed that of homosexual contact. For females, the majority of cases continued to be those

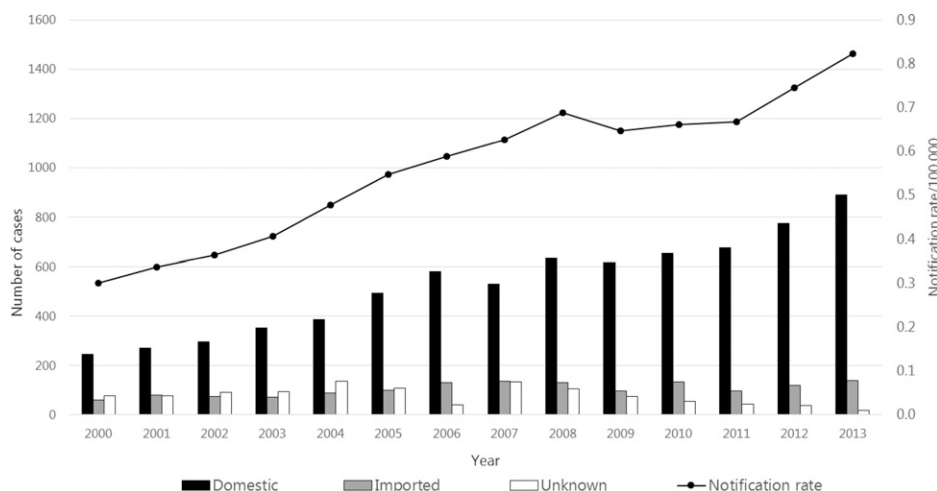


FIGURE 1. Number of reported domestic and imported cases of amebiasis and notification rate per 100,000 in Japan, 2000–2013 (*N* = 9,946). Bars express number of cases from suspected area of infection: black indicates domestic cases, gray imported cases, and white unknown cases. Line expresses notification rate/100,000 of amebiasis.

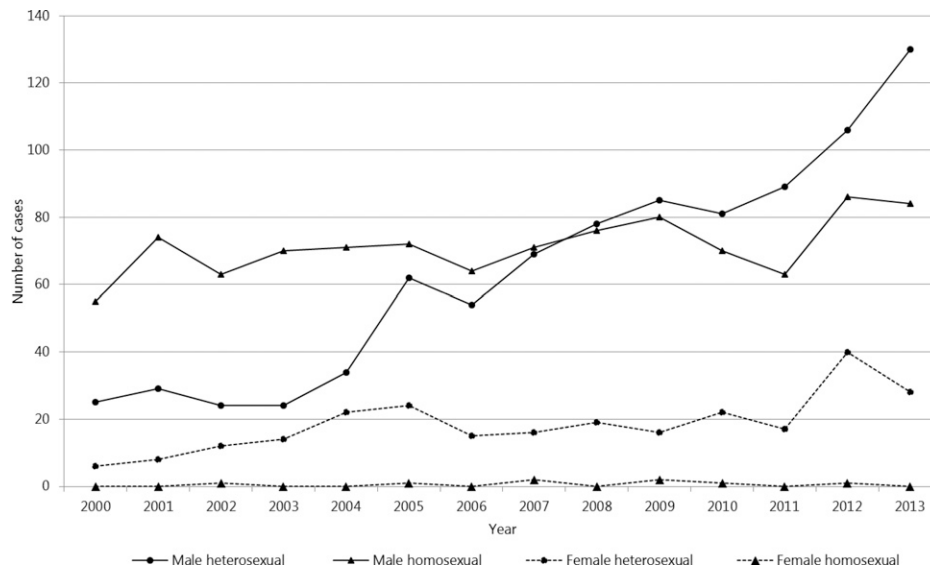


FIGURE 2. Number of reported domestically acquired amebiasis among cases with suspected sexual route of infection in Japan, 2000–2013 ($N = 2,156$). Solid line indicates male cases and dotted line female cases. Circle indicates heterosexual route of infection and triangle homosexual route of infection.

associated with heterosexual contact throughout the study period (Figure 2).

Description of domestic cases between 2010 and 2013.

Given the rapid increase in domestic cases in 2012 and 2013, we described the distribution of characteristics between 2010 and 2013 (Table 1). For both male and female cases, more than half of reported cases during 2010–2013 were from urban areas of Metropolitan Tokyo, Osaka, Kanagawa, Aichi, and Chiba prefectures, comprising 51.5% (1,541/2,994) of the total reported cases. The proportion of cases aged 50 years or older increased slightly from 44.0% to 49.8% during 2010–2013. The majority of cases were male (approximately 87.0%) throughout this period (Table 1).

During 2010–2013, the proportion of asymptomatic cases increased from 6.0% to 19.1% and the proportion of cases diagnosed by colonoscopy increased from 65.0% to 73.1% (Table 1). The proportion of male cases with unknown route of infection also increased from 48.9% in 2010 to 54.3% in 2013. Among male cases attributed to sexual activity, the proportion attributed to heterosexual contact increased over the period from 43.5% to 50.4%. The proportion of females with unknown route of infection ranged from 45.3% in 2012 to 57.6% in 2011. The proportion acquired sexually ranged from 22.4% in 2011 to 40.6% in 2012, majority of which were heterosexual (Table 1).

Analysis of asymptomatic and symptomatic cases. A higher proportion of female cases were symptomatic (356/379 [93.9%]) compared with males (2,245/2,615 [85.8%]) and the proportion of cases < 50 years of age in females (289/379 [76.3%]) was higher than males (1,311/2,615 [50.1%]). For both males and females, comparison of asymptomatic and symptomatic domestic cases from 2010 to 2013 showed that asymptomatic status was significantly more likely to be diagnosed by colonoscopy (Table 2).

In univariate analysis, among male cases, asymptomatic cases were significantly associated with heterosexual contact, nonsexual contact, and unknown route of infection compared

with those with homosexual contact. Among female cases, majority of symptomatic cases were associated with heterosexual contact, with only two female cases associated with homosexual contact (Table 2). Among male cases, multivariate analysis including age, colonoscopy, and route of infection (homosexual, heterosexual, sexual unspecified, nonsexual, and unknown route of infection) showed that colonoscopy ($OR = 31.5$; 95% $CI = 14.0$ –71.0) and cases with unknown route of infection, compared with those with homosexual contact ($OR = 2.2$; 95% $CI = 1.3$ –3.9), remained associated with asymptomatic infections (Table 2).

DISCUSSION

The reported number of domestically acquired cases of amebiasis increased considerably between 2000 and 2013. During 2010–2013, the proportion of reported cases from urban areas was high, as reported previously.¹⁴ Much of the recent rise in notifications was associated with increase among asymptomatic cases, those diagnosed by colonoscopy, and males associated with heterosexual or unknown route of infection. Moreover, among males, who continued to make up the majority of the cases, performing colonoscopy at diagnosis and those with unknown route of infection were significantly associated with asymptomatic status in multivariate analysis. Although MSM have been considered to be at a higher risk of being identified with amebiasis than heterosexual male cases,^{14,24} the large number of heterosexual male cases in recent years was not previously known and poses a potentially important public health concern. Because oral–anal sex, adjusted for MSM status, has been associated with amebiasis,²⁴ heterosexual male cases may be acquiring infection by oral–anal sex with females.

Although few, case reports of amebiasis among females, including commercial sex workers, have been increasingly reported in Japan.^{12,13,15,25} In fact, symptomatic female cases associated with heterosexual contact increased during the

TABLE 1
Characteristics in domestic cases of amebiasis in Japan, 2010–2013

	2010		2011		2012		2013	
	N = 655		N = 675		N = 775		N = 889	
	n	%	n	%	n	%	n	%
Age (years)								
< 50	367	56.0	342	50.5	419	54.1	446	50.2
≥ 50	288	44.0	334	49.5	356	45.9	443	49.8
Sex								
Female	80	12.2	85	12.6	106	13.7	108	12.1
Male	575	87.8	590	87.4	669	86.3	781	87.9
Symptoms*								
No	39	6.0	77	11.4	107	13.8	170	19.1
Yes	616	94.0	598	88.6	668	86.2	719	80.9
Performed colonoscopy at diagnosis								
No	229	35.0	232	34.4	263	33.9	239	26.9
Yes	426	65.0	453	65.6	512	66.1	650	73.1
Amebiasis type								
Extraintestinal amebiasis†	112	17.1	131	19.4	132	17.0	128	14.4
Intestinal amebiasis	543	82.9	544	80.6	643	83.0	761	85.6
Route of infection among male cases								
Nonsexual	108	18.8	85	14.4	100	14.9	99	12.7
Sexual	186	32.3	194	32.9	234	35.0	258	33.0
Unknown	281	48.9	311	52.7	335	50.1	424	54.3
Route of infection among male cases attributed to sexual activity								
Heterosexual	81	43.5	89	45.9	106	45.3	130	50.4
Homosexual‡	70	37.6	63	32.5	86	36.8	84	32.6
Unspecified	35	18.8	42	21.6	42	17.9	44	17.1
Route of infection among female cases								
Nonsexual	11	13.8	17	20.0	15	14.2	19	17.6
Sexual	24	30.0	19	22.4	43	40.6	34	31.5
Unknown	45	56.3	49	57.6	48	45.3	55	50.9
Route of infection among female cases attributed to sexual activity								
Heterosexual	22	91.7	17	89.5	40	93.0	28	82.4
Homosexual	1	4.2	0	0.0	1	2.3	0	0.0
Unspecified	1	4.2	2	10.5	2	4.7	6	17.6

* Cases with specific colonic mucosa lesions, such as erythema, edematous mucosa, erosion, white exudates, and ulcers, but otherwise asymptomatic and reported through National Epidemiological Surveillance of Infectious Diseases, were classified as asymptomatic cases.

† Extraintestinal cases include cases diagnosed with both intestinal and extraintestinal amebiasis.

‡ Cases marked with both heterosexual and homosexual contact were included in the homosexual category (three in 2010, four in 2011, six in 2012, and three in 2013).

study period (Tables 1 and 2, Figure 2). However, the absolute risk of amebiasis for females appears to be lower, as predominance of male amebiasis cases has been reported consistently from various locations that differ culturally, racially, and socio-

economically,^{8,10} and females appear to be less affected by amebiasis (e.g., liver abscess),^{26,27} which has also been found in animal studies.²⁸ Moreover, while a higher proportion of males in their 50s are screened for colon cancer during annual

TABLE 2
Comparison of characteristics between asymptomatic and symptomatic cases of amebiasis in Japan, 2010–2013

	Male (N = 2,615)						Female (N = 379)					
	Asymptomatic* (N = 370)		Symptomatic (N = 2,245)		OR (95% CI)	Adjusted OR§ (95% CI)	Asymptomatic* (N = 23)		Symptomatic (N = 356)		OR (95% CI)	Adjusted OR§ (95% CI)
	n	%	n	%			n	%	n	%		
Age (years)												
< 50	170	45.9	1,141	50.8	Reference	Reference	17	73.9	272	76.4	Reference	Reference
≥ 50	200	54.1	1,131	50.4	1.2 (0.9–1.4)	1.3 (0.9–2.0)	6	26.1	84	23.6	1.1 (0.4–3.0)	1.3 (0.5–3.5)
Performed colonoscopy at diagnosis												
No	6	1.6	806	35.9	Reference	Reference	1	4.3	150	42.1	Reference	Reference
Yes	364	98.4	1,439	64.1	34.0 (15.1–76.5)	31.5 (14.0–71.0)	22	95.7	206	57.9	16.0 (2.1–120.1)	16.2 (2.1–123.4)
Route of infection among all cases†												
Sexual; homosexual‡	16	4.3	287	12.8	Reference	Reference	0	0.0	2	0.6	–	–
Sexual; heterosexual	44	11.9	362	16.1	2.2 (1.2–3.9)	1.2 (0.7–2.3)	1	4.3	106	29.8	–	–
Sexual; unspecified	15	4.1	148	6.6	1.8 (0.9–3.8)	1.2 (0.5–2.5)	1	4.3	10	2.8	–	–
Nonsexual	53	14.3	339	15.1	2.8 (1.6–5.0)	1.5 (0.8–2.8)	5	21.7	57	16.0	–	–
Unknown	242	65.4	1,109	49.4	3.9 (2.3–6.6)	2.2 (1.3–3.9)	16	69.6	181	50.8	–	–

OR = odds ratio; CI = confidence interval.

* Cases with specific colonic mucosa lesions, such as erythema, edematous mucosa, erosion, white exudates, and ulcers, but otherwise asymptomatic and reported through National Epidemiological Surveillance of Infectious Diseases, were classified as asymptomatic cases.

† Because of sparse cells, estimates for females could not be reliably estimated.

‡ Cases marked with both heterosexual and homosexual contact were included in the homosexual category (one in asymptomatic and 15 in symptomatic).

§ Adjusted for each other for variables listed (age, colonoscopy, and route of infection).

health checks in Japan (41.4% for male and 34.2% for female in 2013),²⁹ this differential screening proportion alone likely cannot explain the much larger difference consistently observed between males and females and the gender difference that has been previously reported.^{1–3,8–10,14,27,28} The specific reason(s) for the observed gender difference in the Japanese surveillance data requires further investigation.

Colonoscopy and biopsy can be helpful in the diagnosis of intestinal amebiasis, especially in less severe cases, but diagnosis by colonoscopy is limited because it is an invasive procedure. In Japan, colonoscopy is performed not only for diagnosis of amebiasis but also for screening of colon cancer for annual health checks and follow-up of inflammatory bowel disease.^{18,30} Recently, the endoscopic procedure has been cited as a potential tool for early diagnosis of amebiasis and for prevention of serious complications.¹⁷

This study, based on nationally reported surveillance data, has several limitations. First, information regarding route of infection were self-reported surveillance data, and self-reported sexual activity information may be inaccurate.³¹ Although 50% of cases had unknown route of infection, the “unknown” classification was selected by the reporting physician from a checkbox. The reason for such a selection may be due to the following reasons: 1) the physician did not ask the patient, 2) the patient had a suspected route in mind but did not answer the question, and 3) the patient had no idea of the potential route of infection. Because the suspected route of infection relates to sexual behavior, the high proportion of the “unknown” category may have been affected by social desirability bias. When considering the flow of clinical referrals for an asymptomatic patient detected by colonoscopy at screening settings, such bias from the physician side seems less likely. This is because such asymptomatic patients with a confirmation of amebiasis from screening would simply be referred to specialists, who would most likely be the same clinicians who see symptomatic amebiasis patients. Another possible reason for the higher proportion of unknown route of transmission for asymptomatic cases may be due to recall bias—asymptomatic cases may have more difficulty recalling a possible route of infection compared with symptomatic cases, and such bias may exist differentially between the two groups. Second, it is unknown if there have been any changes in awareness, practice, or reporting for colonoscopy during the study period. Although laboratory criteria or diagnosis method for notification have not changed,^{12,13} due to improved formatting of the notification form in February 2011, indicating “colon lesion” may have become easier, possibly leading to a greater chance of a case of amebiasis with only colon lesion (detected by colonoscopy) to be notified. Finally, confirmation of amebiasis based on microscopy was greater among cases diagnosed by colonoscopy (95%) relative to those diagnosed by methods other than colonoscopy (50%). This may be a potential concern as *E. dispar* cannot be morphologically differentiated from *E. histolytica*, a species that also parasitizes human intestines but is known to be non-pathogenic. In a sub-analysis restricted to 825 cases confirmed by molecular techniques (i.e., PCR, $N = 44$; serology, $N = 770$; ELISA, $N = 25$; ELISA and PCR, $N = 2$; ELISA and serology, $N = 7$; and PCR and serology, $N = 5$), where the specificity for *E. histolytica* is high with lower potential for false positives due to *E. dispar*, overall similar results were obtained. The restricted analysis showed an increase in noti-

fications from 184 cases in 2010 to 240 in 2013, indicating 1.4-fold increase in notified cases (1.3-fold increase for total cases). In addition, the sex and age distribution were similar; the majority of cases were males (ranging from 82.7% to 89.2%) and the proportion of cases aged 50 years or older increased slightly from 44.0% to 50.0% during 2010–2013. Furthermore, while the proportion of asymptomatic cases among the restricted cases was lower than that of the total cases, there was an increase in both the number and proportion of asymptomatic cases from 2010 to 2013 (6/184 (3.3%) in 2010 to 17/240 (7.1%) in 2013). Similarly, while the proportion of restricted cases with colonoscopy was considerably lower than that of total cases, there was an increase in both the number and proportion of colonoscopy cases from 2010 to 2013 (51/184 (27.7%) in 2010 to 99/240 (41.3%) in 2013). It is expected that total cases, which included those detected by microscopy, would have a higher proportion of asymptomatic cases and those detected by colonoscopy, because most asymptomatic cases were detected by colonoscopy (Table 2), and such cases would usually be diagnosed by microscopy rather than molecular methods in the clinical setting. Similarly, males with unknown or heterosexual route of infection increased in both the number and proportion of cases from 2010 to 2013 (unknown: 72 (45.6%) in 2010 to 105 (50.2%) in 2013; heterosexual: 19 (32.8%) in 2010 to 35 (40.7%) in 2013). Furthermore, among males, colonoscopy remained significantly associated with asymptomatic infections in multivariate analysis (OR = 106.8; 95% CI = 14.6–780.0). And, when assessing routes of infection relative to homosexual contact, cases with unknown route of infection was significantly associated with asymptomatic status in univariate analysis (OR = 5.4; 95% CI = 1.3–23.0), although the association was not significant in multivariate analysis (OR = 2.7; 95% CI = 0.6–12.5). Therefore, despite smaller sample numbers, our results from the sub-analysis were similar to our main findings. Moreover, *E. histolytica* has been consistently found to be the predominant species isolated in Japan, with *E. dispar* being very rare.^{22,32} Such apparent lack of *E. dispar* in Japan appears to be a globally unique phenomenon. Thus, although majority of cases diagnosed by colonoscopy were due to microscopy, given the predominance of *E. histolytica* in Japan, and the similar results obtained from the sub-analysis restricting to cases diagnosed by molecular methods, misclassification due to *E. dispar* seems unlikely to play a role. However, studies to indicate the presence of *E. histolytica* and lack of *E. dispar* among Japanese amebiasis cases reported through surveillance may be helpful to confirm previously reported findings.

Our study is the first epidemiological study of domestically acquired amebiasis in Japan using national surveillance data. These results showed that the main increase in recent reports of amebiasis were associated with asymptomatic male cases diagnosed by colonoscopy, with either “unknown” or heterosexual route of transmission. Although the recent rise may be due to enhanced detection by colonoscopy or reporting of such cases, this study has led to a better understanding of amebiasis in Japan and highlights the potential public health concern, as indicated by the large number of asymptomatic male cases, with hitherto unreported routes of infection. It is thus important to communicate these findings to clinicians and improve the quality of surveillance data being collected, particularly regarding the suspected route of infection.

Through dissemination of these epidemiological data with the clinical community, further studies, such as those focusing on identifying the potential route(s) of infection among asymptomatic male cases diagnosed by colonoscopy (e.g., sexual partnerships and specific sexual activities) and observational epidemiologic studies that focus on potential risk factors, may shed light on the current situation. With improved understanding, risk groups may be identified to effectively and efficiently control and prevent the spread of amebiasis in Japan.

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Authors' addresses: Masahiro Ishikane, Field Epidemiology Training Program, National Institute of Infectious Diseases, Tokyo, Japan, and Division of Global Infectious Diseases, Department of Infection and Epidemiology, Graduate School of Medicine, Tohoku University, Miyagi, Japan, E-mail: ishikane@niid.go.jp. Yuzo Arima, Infectious Disease Surveillance Center, National Institute of Infectious Diseases, Tokyo, Japan, E-mail: arima@niid.go.jp. Atsuhiko Kanayama, Field Epidemiology Training Program, National Institute of Infectious Diseases, Tokyo, Japan, and Department of Global Infectious Diseases and Tropical Medicine, National Defense Medical College, Saitama, Japan, E-mail: kanayama@ndmc.ac.jp. Takuri Takahashi, Takuya Yamagishi, Yuichiro Yahata, Tamano Matsui, Tomimasa Sunagawa, and Kazunori Oishi, Infectious Disease Surveillance Center, National Institute of Infectious Diseases, Tokyo, Japan, E-mails: takuri@niid.go.jp, tack73@gmail.com, yahata@niid.go.jp, djiyu@niid.go.jp, sunatomi@niid.go.jp, and oishik@niid.go.jp. Tomoyoshi Nozaki, Department of Parasitology, National Institute of Infectious Diseases, Tokyo, Japan, E-mail: nozaki@niid.go.jp.

Reprint requests: Yuzo Arima, Infectious Disease Surveillance Center, National Institute of Infectious Diseases, 1-23-1 Toyama, Shinjuku-ku, Tokyo 162-8640, Japan, E-mail: arima@niid.go.jp, Tel: +81-3-5285-1111, Fax: +81-3-5285-1233.

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