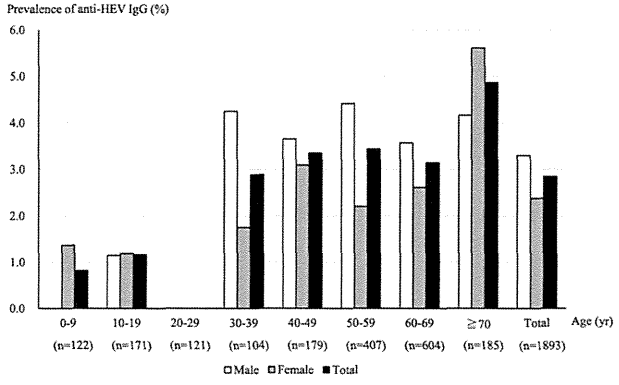

Fig. 1. The age- and sex-specific prevalence of anti-HEV IgG class antibody in liver transplant recipients in Japan. The overall prevalence of anti-HEV IgG class antibody in liver transplant recipients was 2.9% (male, 3.3%; female, 2.4%). The prevalence generally increased with age.. n: the number of the recipients in each age group.

recurrence of hepatitis B and C, and seemed unrelated to HEV infection. In addition, even in the ongoing HEV infection cases, liver dysfunction was not severe or within normal limit sometimes. These laboratory data fluctuates easily in liver transplant recipients and it should be difficult to suspect active HEV infection from the clinical features alone.

In recent years, the prevalence and clinical courses of hepatitis E in transplant recipients were reported in European countries, North America and Iran (Legrand-Abravanel et al., 2010; Halac et al., 2012). In a review by Zhou et al. (2013), the overall prevalence of anti-HEV IgG class antibody in SOT recipients was 11.6% and 7.4% in liver transplant recipients. This review article included the results detected by several different ELISA kit, and different commercial serological measurements are known to indicate a variability of the prevalence rate (Kamar et al., 2012). Khan et al. (2011) analyzed the sensitivity and specificity of four ELISA systems for HEV antibodies (Abbott, Cosmic, TGH and Wantai) and revealed that Wantai test had the highest sensitivity and specificity (100% and 100%, respectively) among them. In this report, Cosmic test using our ELISA system also showed high sensitivity and specificity (98.1% and 100%, respectively) following Wantai test. Although it is difficult to compare, of course, the prevalence of IgG antibody in our study (2.9%) was lower than these reviewed prevalence rates. This is due to the intrinsic lower prevalence of HEV in the general population in Japan compared with in European countries (Kamar et al., 2012; Takahashi et al., 2010a). The prevalence in liver transplant recipients in Japan was also lower than 5.1% in Japanese blood donors whose age was 40–59 years old, and the male rate was 50% (Takeda et al., 2010). We have reported the prevalence of healthy people (Takahashi et al., 2010a), blood donors (Gotanda et al., 2007), dialysis patients (Mitsui et al., 2004) in Japan using the same ELISA method in this study, and the prevalence of Japanese recipients is also lower than these previous results. The low prevalence in the recipients is not thought to arise from the sensitivity of the measurement but from the special characteristics of the patients in this study.

Table 3
Characteristics of HEV RNA-positive liver transplant recipients.

Variables	Case 1	Case 2
Age [years], sex	60, female	41, male
Living area	Kanto	Kyushu
Primary disease	Primary sclerosing cholangitis	Non-alcoholic steatohepatitis, hepatocellular carcinoma
Time from transplantation [months]	8	3
Laboratory data at sampling		
WBC [/μL]	1500	6170
Lymphocyte [/μL]	453	882
AST [IU/L]	23	36
ALT [IU/L]	27	48
T-Bil [mg/dL]	0.8	0.4
γ-GT [IU/L]	81	63
Immunosuppression	Tacrolimus, corticosteroids	Tacrolimus, corticosteroids
Liver injury episode after transplantation	+	+
HEV genotype	3	3

The conceivable causes of the lower prevalence in the transplant recipients are as follows: First, it is possible that the reduction of the anti-HEV antibody titers was induced by immunosuppressive drugs (Sester et al., 2008). Second, avoiding consumption of raw or undercooked meat or shellfish after transplantation might reduce the chance of HEV infection, as normally seen in the general population. At 11 of the 17 institutions in this survey, the liver transplant recipients were prohibited from consuming raw or undercooked foods for at least 6 months after transplantation. Unfortunately, the serum samples before transplantation were not available from the majority of the studied patients, except for those from Case 1 and 2 patients with HEV viremia, and the prevalence of antibodies before and after transplantation could not be compared. However, on the basis of the assumption that half of acute HEV infections progress to chronic phase in transplant recipients (Behrendt et al., 2014), the liver transplant patients in Japan had less opportunity to be infected. In the two of HEV infection cases, IgA antibodies were not detected throughout the following course. Although anti-HEV IgA antibody measurement is covered by insurance in Japan because of its high sensitivity and specificity (Takahashi et al., 2005), HEV RNA detection seems to be the most suitable to detect the chronic infection in immunocompromised patients. The methods used in this study present a sufficient sensitivity and the low presence of ongoing HEV infection is likely to be true.

HEV infection is usually self-limiting in immunocompetent individuals and therefore specific therapies are not required in the majority of cases. However, it can lead to chronic hepatitis and liver cirrhosis in patients taking immunosuppressants after organ transplantation. Haagsma et al. (2009) reported two cases of liver transplantation in which chronic HEV infection developed gradually into graft cirrhosis and a second transplantation was needed. To prevent graft failure, persistent HEV infection in liver transplant recipients should not be overlooked and untreated. The first strategy for recipients with

Table 4
The behavior of anti-HEV antibodies and HEV RNA in the chronic infection cases.

	Case 1					Case 2			
	Anti-HEV IgG [OD value]	Anti-HEV IgM [OD value]	Anti-HEV IgA [OD value]	HEV RNA [copies/mL]		Anti-HEV IgG [OD value]	Anti-HEV IgM [OD value]	Anti-HEV IgA [OD value]	HEV RNA [copies/mL]
Pre-transplantation	0.410 (+)	0.041 (−)	0.062 (−)	–	*Pre-transplantation*	0.016 (−)	0.051 (−)	0.197 (−)	–
At diagnosis (255 POD)	>3.000 (+)	0.223 (−)	0.089 (−)	8.1×10^5	At diagnosis (81 POD)	0.204 (+)	1.063 (+)	0.082 (−)	1.3×10^6
After diagnosis (360 POD)	>3.000 (+)	0.203 (−)	0.105 (−)	5.5×10^4	After diagnosis (249 POD)	>3.000 (+)	1.480 (+)	0.143 (−)	3.2×10^6
Liver donor	0.021 (−)	0.029 (−)	0.021 (−)	–	Liver donor	0.006 (−)	0.036 (−)	0.010 (−)	–
Blood donor (FFP)	NA	NA	NA	+[a]	Blood donor (Platelet)	NA	NA	NA	1.8×10^4

Note: OD, optical density; POD, postoperative day; FFP, fresh frozen plasma; NA, not available.
[a] The titer of HEV RNA was not available.

persistent HEV infection is considered to be reduction of the immunosuppressants, the second strategy is considered to be anti-viral therapies (Kamar et al., 2011). In our study, Case 1 patient had received anti-viral therapy and Case 2 had been followed up without any anti-viral therapy or reduction of the immunosuppressants after the diagnosis. More detailed information about the clinical courses of these cases will be published in a short time.

A previous study on the risk factors in SOT recipients for developing chronic hepatitis after acute HEV infection revealed that the recipients who developed chronic hepatitis had significantly lower counts of leukocytes, total lymphocytes, platelets, and CD2, CD3, and CD4 lymphocytes than transient patients (Kamar et al., 2008). In the present study, two cases with chronic infection were exposed to HEV in the early postoperative period and lower counts of lymphocytes in peripheral blood were observed. The reduced production of antibodies due to high dose immunosuppressants in the early postoperative period could contribute to the development of chronic infection even in a patient previously exposed to HEV. However, we cannot easily conclude from our results that these are the factors for risk of a chronic career after acute HEV infection, because we could neither detect any transient hepatitis patient nor compare the progress of acute and chronic infections.

HEV genotypes 3 and 4 usually cause sporadic infections, in industrialized countries most likely due to intake of contaminated food. HEV RNA has been detected in a variety of food products, especially in porcine livers (Colson et al., 2010). However, HEV can also be transmitted via the transplanted organ itself or through blood transfusion. Schlosser et al. reported that HEV transmission via the infected donor liver lead to chronic infection and graft cirrhosis development after liver transplantation (Schlosser et al., 2012). Several previous case reports suggested that HEV transmission by blood products is possible (Matsubayashi et al., 2004; Wedemeyer et al., 2012). According to the newest epidemiology of autochthones hepatitis E virus infection in Japan, 11 patients of 43 asymptomatic hepatitis E cases were detected from blood donors (Kanayama et al., 2015). In another report, 2% of HEV infections are ascribable to blood transfusion in Japan (Abe et al., 2006). Recently, Hewitt et al. reported that 42% of the patients in England transfused with HEV-positive blood products had evidence of infection with HEV and immunocompromised patients, including organ transplant recipients, showed prolonged viral infection (Hewitt et al., 2014). The two patients reported in our present study were the first cases with transfusion-transmitted chronic hepatitis E in liver transplant recipients in Japan, to the best of our knowledge. In addition, de novo HEV infection developed despite the presence of anti-HEV antibodies in Case 1. Takahashi et al. reported that the majority of HEV virions in serum possess lipids on their surfaces and inhibit neutralization by antibodies (Takahashi et al., 2010b). In Japan, screening of donated blood for HEV has only been conducted in the Hokkaido area, where HEV infection is most prevalent (Takahashi et al., 2010a). Patients receiving blood products include many immunocompromised individuals, so screening of blood donors for HEV in other areas in Japan is probably desirable: moreover, further investigations about HEV infection in other transplant recipients and immunosuppressed patients are required.

5. Conclusions

This study is the first multicenter survey for HEV infection in Asian liver transplant recipients and the presence of HEV infection is low in Japan; however, liver transplant recipients have a risk of transfusion-transmitted chronic hepatitis E. HEV infection should be considered as a differential diagnosis and molecular biological detection of HEV should be essential, when the recipient receives blood products and presents unknown liver injury.

Author Contributions

Yuki Inagaki: literature search, study design, patients' samples and data collection, data analysis, data interpretation, writing, tables and figures.

Yukio Oshiro: literature search, study design, participating institution recruitment, data interpretation, writing.

Tomohiro Tanaka, Tomoharu Yoshizumi, Hideaki Okajima, Kohei Ishiyama, Chikashi Nakanishi, Masaaki Hidaka, Hiroshi Wada, Taizo Hibi, Kosei Takagi, Masaki Honda, Kaori Kuramitsu, Hideaki Tanaka, Taiji Tohyama, Toshihiko Ikegami, Satoru Imura, Tsuyoshi Shimamura, Yoshimi Nakayama, Taizen Urahashi, Ken Shirabe, and Norihiro Kokudo: patients' samples and data collection.

Kazumasa Yamagishi: study design, data analysis.

Hiroshi Ohnishi, Shigeo Nagashima, and Masaharu Takahashi: samples measurement, data interpretation.

Hiroaki Okamoto: study design, samples measurement, data interpretation.

Nobuhiro Ohkohchi: study design, participating institution recruitment, data interpretation, corresponding author.

Yuki Inagaki and Yukio Oshiro contributed equally to the preparation of the text. All the authors reviewed and approved this manuscript.

Conflicts of interests

All authors report no conflict of interest.

Acknowledgments/Funding

This study was supported in part by a grant from the Ministry of Health, Labour and Welfare, Japan (H24-Hepatitis-General-002). The grant supported collecting the patients' samples and clinical data, and the measurement of the samples.

References

Abe, T., Aikawa, T., Akahane, Y., Arai, M., Asahina, Y., Atarashi, Y., et al., 2006. Demographic, epidemiological, and virological characteristics of hepatitis E virus infections in Japan based on 254 human cases collected nationwide. Kanzo 8, 384–391 (in Japanese).

Behrendt, P., Steinmann, E., Manns, M.P., Wedemeyer, H., 2014. The impact of hepatitis E in the liver transplant setting. J. Hepatol. 61, 1418–1429.

Colson, P., Borentain, P., Queyriaux, B., Kaba, M., Moal, V., Gallian, P., et al., 2010. Pig liver sausage as a source of hepatitis E virus transmission to humans. J. Infect. Dis. 202, 825–834.

Dalton, H.R., Bendall, R.P., Keane, F.E., Tedder, R.S., Ijaz, S., 2009. Persistent carriage of hepatitis E virus in patients with HIV infection. N. Engl. J. Med. 361, 1025–1027.

Datta, R., Panda, S.K., Tandon, B.N., Madnagopalan, N., Bose, S.L., Acharya, S.K., et al., 1987. Acute sporadic non-A, non-B viral hepatitis of adults in India: epidemiological and immunological studies. J. Gastroenterol. Hepatol. 2, 333–345.

Gotanda, Y., Iwata, A., Ohnuma, H., Yoshikawa, A., Mizoguchi, H., Endo, K., et al., 2007. Ongoing subclinical infection of hepatitis E virus among blood donors with an elevated alanine aminotransferase level in Japan. J. Med. Virol. 79, 734–742.

Haagsma, E.B., Niesters, H.G., van den Berg, A.P., Riezebos-Brilman, A., Porte, R.J., Vennema, H., et al., 2009. Prevalence of hepatitis E virus infection in liver transplant recipients. Liver Transpl. 15, 1225–1228.

Halac, U., Béland, K., Lapierre, P., Patey, N., Ward, P., Brassard, J., et al., 2012. Chronic hepatitis E infection in children with liver transplantation. Gut 61, 597–603.

Hewitt, P.E., Ijaz, S., Brailsford, S.R., Brett, R., Dicks, S., Haywood, B., et al., 2014. Hepatitis E virus in blood components: a prevalence and transmission study in southeast England. Lancet 384, 1766–1773.

Inoue, J., Takahashi, M., Yazaki, Y., Tsuda, F., Okamoto, H., 2006. Development and validation of an improved RT-PCR assay with nested universal primers for detection of hepatitis E virus strains with significant sequence divergence. J. Virol. Methods 137, 325–333.

Kamar, N., Bendall, R., Legrand-Abravanel, F., Xia, N.S., Ijaz, S., Izopet, J., et al., 2008. Hepatitis E virus and chronic hepatitis in organ-transplant recipients. N. Engl. J. Med. 358, 811–817.

Kamar, N., Bendall, R., Legrand-Abravanel, F., Xia, N.S., Ijaz, S., Izopet, J., et al., 2012. Hepatitis E. Lancet 379, 2477–2488.

Kamar, N., Garrouste, C., Haagsma, E.B., Garrigue, V., Pischke, S., Chauvet, C., et al., 2011. Factors associated with chronic hepatitis in patients with hepatitis E virus infection who have received solid organ transplants. Gastroenterology 140, 1481–1489.

Kanayama, A., Arima, Y., Yamagishi, T., Kinoshita, H., Sunagawa, T., Yahata, Y., et al., 2015. Epidemiology of domestically acquired hepatitis E virus infection in Japan: assessment of the nationally reported surveillance data, 2007–2013. J. Med. Microbiol. 64, 752–758.

Khan, A., Tanaka, Y., Kurbanov, F., Elkady, A., Abbas, Z., Azam, Z., et al., 2011. Investigating an outbreak of acute viral hepatitis caused by hepatitis E virus variants in Karachi, South Pakistan. J. Med. Virol. 83, 622–629.

Legrand-Abravanel, F., Kamar, N., Sandres-Saune, K., Garrouste, C., Dubois, M., Mansuy, J.M., et al., 2010. Characteristics of autochthonous hepatitis E virus infection in solid-organ transplant recipients in France. J. Infect. Dis. 202, 835–844.

Matsubayashi, K., Nagaoka, Y., Sakata, H., Sato, S., Fukai, K., Kato, T., et al., 2004. Transfusion-transmitted hepatitis E caused by apparently indigenous hepatitis E virus strain in Hokkaido, Japan. Transfusion 44, 934–940.

Mitsui, T., Tsukamoto, Y., Yamazaki, C., Masuko, K., Tsuda, F., Takahashi, M., et al., 2004. Prevalence of hepatitis E virus infection among hemodialysis patients in Japan: evidence for infection with a genotype 3 HEV by blood transfusion. J. Med. Virol. 74, 563–572.

Mizuo, H., Suzuki, K., Takikawa, Y., Sugai, Y., Tokita, H., Akahane, Y., et al., 2002. Polyphyletic strains of hepatitis E virus are responsible for sporadic cases of acute hepatitis in Japan. J. Clin. Microbiol. 40, 3209–3218.

Okamoto, H., 2007. Genetic variability and evolution of hepatitis E virus. Virus Res. 127, 216–228.

Ollier, L., Tieulie, N., Sanderson, F., Heudier, P., Giordanengo, V., Fuzibet, J.G., et al., 2009. Chronic hepatitis after hepatitis E virus infection in a patient with non-Hodgkin lymphoma taking rituximab. Ann. Intern. Med. 150, 430–431.

Schlosser, B., Stein, A., Neuhaus, R., Pahl, S., Ramez, B., Krüger, D.H., et al., 2012. Liver transplant from a donor with occult HEV infection induced chronic hepatitis and cirrhosis in the recipient. J. Hepatol. 56, 500–502.

Sester, M., Gärtner, B.C., Girndt, M., Sester, U., 2008. Vaccination of the solid organ transplant recipient. Transplant. Rev. (Orlando) 22, 274–284.

Suzuki, K., Aikawa, T., Okamoto, H., 2002. Fulminant hepatitis E in Japan. N. Engl. J. Med. 347, 1456.

Takahashi, M., Tamura, K., Hoshino, Y., Nagashima, S., Yazaki, Y., Mizuo, H., et al., 2010a. A nationwide survey of hepatitis E virus infection in the general population of Japan. J. Med. Virol. 82, 271–281.

Takahashi, M., Tanaka, T., Takahashi, H., Hoshino, Y., Nagashima, S., Jirintai, et al., 2010b. Hepatitis E virus (HEV) strains in serum samples can replicate efficiently in cultured cells despite the coexistence of HEV antibodies: characterization of HEV virions in blood circulation. J. Clin. Microbiol. 48, 1112–1125.

Takahashi, M., Hoshino, Y., Tanaka, T., Takahashi, H., Nishizawa, T., Okamoto, H., 2008. Production of monoclonal antibodies against hepatitis E virus capsid protein and evaluation of their neutralizing activity in a cell culture System. Arch. Virol. 153, 657–666.

Takahashi, M., Kusakai, S., Mizuo, H., Suzuki, K., Fujimura, K., Masuko, K., et al., 2005. Simultaneous detection of immunoglobulin A (IgA) and IgM antibodies against hepatitis E virus (HEV) is highly specific for diagnosis of acute HEV infection. J. Clin. Microbiol. 43, 49–56.

Takahashi, M., Nishizawa, T., Miyajima, H., Gotanda, Y., Iita, T., Tsuda, F., et al., 2003. Swine hepatitis E virus strains in Japan form four phylogenetic clusters comparable with those of Japanese isolates of human hepatitis E virus. J. Gen. Virol. 84, 851–862.

Takeda, H., Matsubayashi, K., Sakata, H., Sato, S., Kato, T., Hino, S., et al., 2010. A nationwide survey for prevalence of hepatitis E virus antibody in qualified blood donors in Japan. Vox Sang. 99, 307–313.

Tei, S., Kitajima, N., Takahashi, K., Mishiro, S., 2003. Zoonotic transmission of hepatitis E virus from deer to human beings. Lancet 362, 371–373.

The Japanese Liver Transplantation Society, 2014. Registry by the Japanese Liver Transplantation Society. Ishoku 49, 261–274 (in Japanese).

The National Institute of Infectious Diseases, 2003–2013. Infectious Diseases Weekly Report (IDWR). Available from http://www.nih.go.jp/niid/ja/idwr.html.

Wedemeyer, H., Pischke, S., Manns, M.P., 2012. Pathogenesis and treatment of hepatitis E virus infection. Gastroenterology 142, 1388–1397.

Zhou, X., de Man, R.A., de Knegt, R.J., Metselaar, H.J., Peppelenbosch, M.P., Pan, Q., 2013. Epidemiology and management of chronic hepatitis E infection in solid organ transplantation: a comprehensive literature review. Rev. Med. Virol. 23, 295–304.