The Prevalence of Anti-hepatitis E in Occupational Risk Groups

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The seroprevalence of anti-hepatitis E virus (HEV) IgG was investigated by using ELISA commercial anti HEV test kit in 408 healthy adults who lived in central part of Thailand, 168 of which were swine workers, 102 were poultry farmers and 138 were government officers. The overall rate of seroprevalence of IgG anti-HEV was 23.3 % (range 16.7-27.9%). The prevalence of anti-HEV antibodies in government officers was 16.7 % and in subjects from swine workers and poultry farmers who worked in farms for more than 2 years were 27.9 % and 24.5 %, respectively. Although there was no difference in anti-HEV prevalence according to three job categories ($p = 0.06$) and to age groups ($p = 0.4$), but seroreactivity of anti-HEV in swine and poultry farmers were statistically significantly higher than those in officers ($p < 0.01$). From this preliminary study, HEV is supposed to be circulating in the central area of Thailand. It appeared that the probability of exposure and re-infection to HEV are higher in farmers than that in government officers. Poor environmental conditions in farms, occupation and low socioeconomic status might be risk factors in HEV infection.

Keywords: *Agricultural workers' diseases, Hepatitis E virus, Occupational diseases, Prevalence, Risk factors, Swine, Zoonoses*

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The hepatitis E virus (HEV) was identified as recently as 1980 and has caused massive epidemics of acute hepatitis in Asia and outbreaks in Africa. The disease is usually mild, except in pregnant women, who suffer a high fatality rate from fulminant hepatic failure[1]. In developed countries, the prevalence of HEV infection among blood donors ranges from 0.4 to 3.9%[2-4]. These sporadic cases had been identified among travelers from areas where it was endemic[5-7]. Recent seroepidemiological studies showed that swine veterinarians and other pig handlers were at higher risk of HEV infection compared to normal blood donors[8]. Moreover, novel strains of human HEV recovered from hepatitis patients in the USA, Japan and Taiwan have been genetically more closely related to strains of swine HEV from respective countries than to other strains of human HEV[4, 9-12]. Swine HEV infections have now been recognized in pigs in many other countries of the world. Recently, avian hepatitis E virus (avian HEV) identified from a chicken with hepatitis-splenomegaly syndrome in the United States were genetically and antigenically related to human and swine HEVs[13]. The ubiquitous nature of the virus in pigs and the demonstrated ability of cross-species infection raise a potential concern for swine HEV. infection[10]. The discovery of swine and avian HEVs suggests that hepatitis E may be a zoonosis.

HEV is a non-enveloped virus with a single-stranded positive-sense RNA genome of about 7·2 kb, approximately 27-30 nm. in diameter. On the basis of

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comparative phylogenetic analysis, HEV is currently classified as the sole member of the genus *Hepevirus*[14]. Based on nucleotide and protein sequencing of HEV, 4 major genotypes (genotype I-IV) have been described worldwide[15]. Despite the variety of genotypes, only two genotypes of HEV, genotype I and genotype III had been reported to date. Serologic tests cannot distinguish between infections with different HEV genotypes[17]. In Thailand, few studies have addressed the prevalence of HEV infection[1,16]. Subsequently, Thai swine HEV isolates revealed that they all belong to genotype III which are believed to infect across species between human and swine and may be other species as well[15,17]. Therefore, the aim of the present study was to determine the prevalence of HEV antibody among apparently healthy populations with different risks of exposure to viral infections, such as swine workers, poultry farmers and government officers in the central area of Thailand.

Material and Method

Study subjects

A total of 408 samples collected in Nakorn-Nayok and nearby provinces in Thailand were analyzed. These subjects were categorized by occupation into

Serological test

Serum samples were stored at -70°C until tested for antibody. Anti-HEV immunoglobulin G (IgG) was determined by using a commercial HEV (ELISA) test kit according to the manufacturer's instructions (Genelabs Diagnostics, Singapore). All serum samples were tested in duplicate. Briefly, 10 µl of the serum sample was added to each well containing 200 µl of the diluent, and the microplate was incubated for 30 min at 37°C. The microplate was washed six times with 250 µl of a wash solution. Horseradish peroxidase-labeled goat anti-human IgG (Genelabs Diagnostics, Singapore) was used as the conjugates. One hundred microliters of a 1:1,000-diluted conjugate was added to each well, and the microplate was further incubated for 30 min at 37°C. The microplate was washed six times with 250 µl of the wash solution and 100 µl of tetramethylbenzidine substrate (AMRAD Biotech, Melbourne, Australia) was added and incubated for 15 min at room temperature. Then H_2SO_4 was added to stop the reaction and absorbance was read at 450 and 620 nm. The mean of absorbance of three negative controls was determined as cut-off (CO). The absorbance less than cut-off value was considered negative. Samples with absorbance (S) greater than or equal to the CO value were tentatively considered reactive. Results were recorded as S/CO ratios to allow comparison of the intensity reactions of individual samples. The sample was considered reactive when the S/CO ratio was higher than 1.0.

	Swine farmers*	Poultry workers*	Government officers
Male ; female	97 ; 71	61 ; 41	53 ; 85
Total	168	102	138
Age	18-55	18-63	19-60
Median	31	34	36

*All subjects had worked in farms for more than 2 years

Statistical analysis

Statistical comparison of distribution was carried out using the χ^2. For comparison of means and standard deviations, the Kruskal-Wallis (among three occupational groups) and Mann-Whitney test (between two groups) were used. The level of significance was considered at $p < 0.05$.

Results

Occupation-independent prevalence of anti-hepatitis E virus

The overall rate of seroprevalence of IgG anti-HEV was 23.3 % among healthy subjects in the central area of Thailand. The prevalence of HEV infection among three occupational groups in the present study ranged from 16.7-27.9%. The highest prevalence of antibodies to HEV was found in swine workers. Anti-HEV IgG was detected in 23 (16.7%) out of 138 government officers and in 25 (24.5%) out of 102 poultry farmers who had worked in poultry farms for more than 2 years. There was no trend of difference in the prevalence of anti-HEV among these three groups ($p = 0.06$). In addition, there was no significant age-dependent difference in the prevalence of hepatitis E virus ($p = 0.4$). The positive rates of anti-HEV stratified by occupation and age are summarized in Table 1.

Seroreactivity of anti-HEV in each group

The anti-HEV reactivity of sera from these populations was also analyzed. For swine farmers the mean S/CO ratio was 1.22 ± 0.93 (range, 0.2 to 4.35). In poultry farmers the mean S/CO ratio was 1.09 ± 0.77 (range, 0.2 to 3.10). In government officers the mean S/CO ratio was 0.88 ± 0.55 (range, 0.25 to 2.4). The mean S/CO ratios were significant different among these three groups ($p = .021$). The mean S/CO ratio of samples from

Table 1. The seroprevalence of antibodies to hepatitis E virus by occupational groups and ages 9

Occupations	No. positive (%)	Age range (years)					
		18-20 (n = 32)	21-30 (n = 146)	31-40 (n = 133)	41-50 (n = 70)	51-60 (n = 26)	> 60 (n = 1)
swine farmers (n = 168)	47 (27.9%)*	2	14	17	12	2	-
poultry workers (n = 102)	25 (24.5%)*	3	10	8	3	1	-
Gov. officers (n = 138)	23 (16.7%)*	-	9	12	2	-	-
Total (n = 408)	95 (23.3%)	5	33	37	17	3	-
		15.6%**	22.6%**	27.8%**	24.3%**	11.5%**	

* No significant difference among three occupational groups
** No significant difference among all age groups

swine workers (2.63 ± 0.47) was significantly different from those found in poultry farmers (2.34 ± 0.0.38; $p = 0.02$) and in government officers (1.85 ± 0.33; $p < 0.01$). The mean S/CO ratios of positive samples from poultry farmers and government officers were significantly different ($p < 0.01$) as shown in Fig. 1.

Discussion

HEV infection causes acute or chronic liver disease, regardless of living place and lifestyle. In Thailand, hepatitis E virus (HEV) infection is believed to be non-endemic and outbreaks of HEV transmission have never been documented to date. In the present study, serological evidence of HEV now confirms the circulation of HEV in the central area of Thailand. HEV infection was highly prevalent among adults 18 to 65 years of age in central Thailand not only among those living in rural areas but also among people in an urban area. High seroprevalence of IgG anti-HEV antibodies in these sporadic HEV cases suggests that it is unlikely to be protective antibody[18]. The high rate of prevalence of anti-HEV in healthy subjects indicates that subclinical infection may exist[19].

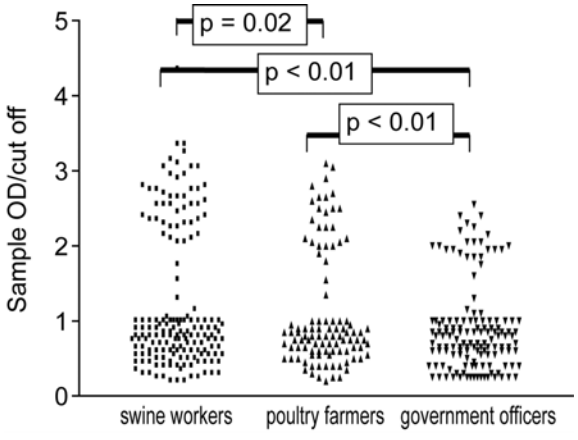

Fig. 1 The reactivity of anti-HEV IgG from serum of three occupational groups

In the present study, seroprevalence ranged from 16.7 % to 27.9 % and higher prevalence of anti-HEV was found in swine farmers and poultry workers, 27.9 and 24.5 respectively. Prevalence of anti-HEV (IgG) was also similar across all adult ages ($p = 0.4$) between the three occupational groups ($p = 0.06$). According to previous reports, the prevalence of anti-HEV in healthy blood donors in Thailand was 15.7%[1]. In this study, the prevalence of anti-HEV did not differ between in government officers (16.7%) and in blood donors. This is inconsistent with Hmong people in the northern Thailand where overall seroprevalence rate of anti-HEV was 6.5%[1,16]. It is not surprising that the prevalence of HEV infection among Hmong people was quite low. Hmong traditionally lived in isolated highlands and the principal occupation of Hmong in Thailand is the intensive cultivation of fruits and vegetables.

No difference in anti-HEV prevalence according to three job categories (swine worker, poultry farmer and government officer) was found in our study. Seroreactivity of anti-HEV in swine workers was significant higher than those in poultry farmers ($p = 0.02$) and in government officers ($p < 0.01$), respectively. To explain the higher amount of anti-HEV in swine and poultry farmers, it appeared that the probability of exposure and re-infection to HEV are higher in farmers than that in government officers. With higher risk of HEV infection in farmers, re-exposure to or re-infection by HEV could occur. Basically, humoral immune responses are more potent in secondary infection. Once infected with HEV, anti-HEV antibodies

are maintained for at least 5 years[20] and wean off with time[21]. Poor environmental conditions in farms and low socioeconomic status might be risk factors in HEV infection. The results suggest that workers in farms may be at somewhat higher risk of HEV infection. Therefore, the studies should be expanded to the domestic pig that have a higher chance of contact with humans to elucidate the infectious source(s) and route(s) for acquiring sporadic hepatitis E in Thailand.

In conclusion, HEV infection as shown by anti-HEV response was found in general population in the central area of Thailand. There was no difference in anti-HEV prevalence according to three job categories. Anti-HEV IgG was distributed almost equally among all age groups. Seroreactivity of anti-HEV in swine workers was significant higher than those in poultry farmers and government officers. The high prevalence rate from the present study emphasized that human and swine HEV genotypes should be investigated.

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ความชุกของแอนติบอดีต่อไวรัสตับอักเสบ อี ในกลุ่มอาชีพเสี่ยง

ภาวิน พัวพงษ์พร, ขวัญจิตร สำราญทรัพย์, พรพรรณ โรจนแสง, สุภาภรณ์ วิวัฒนากุล, สุรางค์รัตน์ ศรีสุรภานนท์

ความชุกของแอนติบอดีชนิด IgG ต่อเชื้อไวรัสตับอักเสบ อี โดยใช้ชุดตรวจสำเร็จรูป โดยวิธี ELISA ในคนปกติจำนวน 408 คน ที่อาศัยอยู่ในภาคกลางของประเทศไทย โดยมีอาชีพเลี้ยงหมู 168 คน คนเลี้ยงไก่ 102 คน และรับราชการ 138 คน พบว่า มีแอนติบอดีต่อเชื้อไวรัสตับอักเสบ อี ร้อยละ 23.3 (ระหว่างร้อยละ 16.7-27.9) โดยที่กลุ่มข้าราชการ พบร้อยละ 16.7 และกลุ่มเลี้ยงหมูและเลี้ยงไก่ ซึ่งมีอาชีพเลี้ยงสัตว์มานานกว่า 2 ปี พบร้อยละ 27.9 และ 24.5 ตามลำดับ แม้ว่าความชุกของแอนติบอดีต่อเชื้อไวรัสตับอักเสบ อี ไม่มีความแตกต่างกันตามอาชีพ (p = 0.06) และอายุ (p = 0.4) แต่พบว่าปฏิกิริยาของค่าแอนติบอดี ในคนเลี้ยงหมูและเลี้ยงไก่มีสูงกว่าคนที่มีอาชีพรับราชการ อย่างมีนัยสำคัญ (p ≤ 0.01) จากการศึกษาเบื้องต้นนี้แสดงว่า เชื้อไวรัสตับอักเสบชนิด อี มีการแพร่กระจายในประชากรภาคกลางของประเทศไทย โดยในกลุ่มคนเลี้ยงสัตว์ปีกและสุกร มีโอกาสเสี่ยงต่อการสัมผัสและติดเชื้อมากกว่ากลุ่มอาชีพรับราชการ ดังนั้น สภาวะของสิ่งแวดล้อม อาชีพการทำงาน และมีเศรษฐานะ อาจเป็นปัจจัยเสี่ยงต่อการติดเชื้อไวรัสตับอักเสบ อี