

## A Special Risk Group for Hepatitis E Infection: The First Record of North Cyprus

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### Abstract

Hepatitis E virus (HEV) is transmitted by a fecal oral route from animals to humans following exposure to the body fluids of infected animals. We investigated the seroprevalence of anti-hepatitis E (anti-HEV) antibodies by monitoring IgG and IgM virus antibodies amongst employees in the animal industry in North Cyprus through a cross-sectional study. Samples were taken from individuals without occupational exposure to animals and from those who worked with animals (doing animal husbandry, veterinary work or butchery). Enzyme-linked immunoassays were used to detect anti-HEV IgG and IgM in the blood samples. The prevalence of anti-HEV IgG antibodies was 3.0% (12/400), while the prevalence of anti-HEV IgM antibodies was 0.25% (1/400). The prevalence of anti-HEV IgG amongst the samples received from females was approximately 2.5-fold higher than samples received from males (2.4%). Anti-HEV IgG was detected amongst 7% of animal husbandry workers and amongst 2% of veterinarians and butchers. The current findings represent the first records of HEV surveillance in Cyprus. We investigated the seroprevalence of anti-HEV by monitoring IgG and IgM virus antibodies amongst employees.

Key words: Cyprus, hepatitis E, seroprevalence, zoonoses, animals, epidemiology

### Introduction

Hepatitis E (HEV) infection involves a non-enveloped virus belonging to the genus of *Orthohepevirus*. It is a zoonotic pathogen which has spread through a number of developing countries (Wu et al. 2016). The virus has been designated with the letter E for its transmission via the enteric route (Lapa et al. 2015). Currently, it is estimated that 20 million individuals worldwide are infected with HEV, where it is the cause of 57 000 deaths each year (Lapinski et al. 2016). It is also responsible for 3.3 million new symptomatic infections with fatal outcomes in 56 600 individuals (Mauceri 2018). The World Health Organization (WHO) has reported that the low endemic regions of HEV epidemiology are found in the USA and Europe (the UK, France, the Netherlands, Austria, Spain, Greece and Germany) as well as developed Asian-Pacific countries (Japan, Taiwan, Korea, Hong Kong, Australia and

New Zealand) (WHO 2010). High endemic regions are found in Central America, Africa and South and Central Asia (Melgaco 2018). The prevalence of anti-HEV IgG in Africa is between 4.6% and 10.7% and is between 34.8% and 94% in Asia (Melgaco 2018).

The presence of anti-HEV IgM was conclusive at the onset of the infection and increased by the beginning of the fourth week; however, no further increase was detected after three months. Anti-HEV IgG antibodies increase to their peak level by the end of the fourth week and can continue for years (El-Tras et al. 2013). HEV is generally transmitted by the fecal-oral route, following transfusion with infected blood products, through vertical transmission and also through contact with infected animals (Hesamizadeh et al. 2016). Individuals considered to be at high risk of contracting the virus include employees of slaughterhouses, people working on pig farms and veterinarians (Holt et al. 2016).

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The aim of the study was to determine the health risks posed to employees working with animals in North Cyprus by monitoring the prevalence of HEV.

## Experimental

### Materials and Methods

**Setting.** The present study was a cross-sectional survey of animal workers from five regions in North Cyprus. The distribution of participants across the different districts in North Cyprus was as follows: Nicosia (28.8%), Kyrenia (18%), Omorphou (11%), Famagusta (21.7%) and Trikomo (20.5%) according to animal workers numbers supplied by Veterinary Office of North Cyprus. Since HEV prevalence may be as low as 1%, taking  $\alpha = 0.05$  and  $\beta = 0.20$  one could calculate the minimum number of people to be investigated as 400. Therefore, four hundred persons were selected who did not have any clinical findings according to general health state. Individuals participating in the study were categorized into four groups based on the level of interaction each participant had with animals in their work life. Group 1 included participants without occupational exposure to animals, while Group 2 consisted of participants who worked with animals (animal husbandry). Group 3 included veterinarians, and Group 4 was composed of butchers. All participants were asked by attained healthy worker to complete epidemiological questionnaire that included basic demographic and epidemiological data such as name, age, sex, how much years to spend as animals' workers.

**Laboratory Testing.** A total of 400 blood samples were collected. Serum samples were separated by centrifugation at  $1000 \times g$  for 10 min and were stored at  $-20^\circ\text{C}$  until required for antibody detection. All serum samples were tested using a commercially available enzyme-linked immunoassay (ELISA) in a kit (Dia Pro, Italy), in accordance with the manufacturer's instructions. The positive and negative controls were supplied in the kit and were included in each antibody screening test. Anti-HEV IgG and anti-HEV IgM ELISA tests were performed separately. The results from all the samples were calculated as a ratio of the individual absorbance to

the cut-off value (S/CO). The samples with a ratio  $< 1.0$  were considered as negative, 1.0–1.2 as equivocal and  $> 1.2$  as positive. The diagnostic sensitivity of  $> 98\%$  was found according to manufacturer's instruction.

**Ethics Committee Approval.** Ethical approval was granted by the Institutional Research Ethics Committee of the Near East University (No. 179).

**Statistical Methods.** The software program SPSS version 3.0 (SPSS Inc. Chicago, IL, USA) was used for the statistical analyses. Student *t*-tests were used for mean differences. Data were expressed as a mean value (standard deviation), as a minimum-maximum and as a percentage, where appropriate. A *p*-value of less than 0.05 was considered statistically significant.

## Results

The mean age of participants was 39.4, with the youngest being 13 and the eldest being 84 years of age (Table I). Three percent of the samples examined were positive for anti-HEV IgG (12/400), while 0.25%

Table I  
The socio-demographic characteristics of groups (n = 400).

Age in years	N (%)
< 30	108 (27.0)
30–40	109 (27.2)
41–50	80 (20.0)
51–60	87 (21.8)
> 61	16 (4.0)

of samples were tested positively for anti-HEV IgM antibodies (1/400) as shown in Table II. The 400 participants consisted of 334 males and 66 females. The prevalence of anti-HEV IgG antibodies was 6.1% (n = 4) amongst female participants and 2.4% (n = 8) amongst male participants (Table II); however, these differences were not statistically significant ( $p = 0.115$ ). Similarly, the anti-HEV IgM seropositivity was not significantly associated with gender ( $p = 0.836$ ). Anti-HEV IgG seropositivity was significantly associated with years spent working with animals ( $p = 0.001$ ). Ten cases of anti-HEV IgG were found for people who spent more

Table II  
Prevalence of the Anti-HEV IgG and IgM seropositivity among gender and time spent in contact with animals.

		Anti-HEV IgG (+) Positive			Anti-HEV IgM (+) Positive		
Female vs. male	Female	Male	<i>p</i> -value	Female	Male	<i>p</i> -value	
	4	8	0.115	0	1	0.836	
Time spent in contact with animals		< 20 years	> 20 years	<i>p</i> -value	< 20 years	> 20 years	<i>p</i> -value
	2	10	0.001	1	0	0.644	

Table III  
Prevalence of the Anti-HEV IgG and IgM seropositivity  
in different locations.

Location	IgG-positive 12 (3%)	IgM-positive 1 (0.25%)
Nicosia	1 (0.01%)	0
Kyrenia	2 (2.7%)	0
Morphou	2 (4.5%)	0
Famagusta	6 (6.9%)	1 (1.5%)
Trikomo	1 (1.2%)	0

than 20 years in contact with animals (Table II). Concerning the geographical localization, of the 12 samples which tested positive for anti-HEV IgG antibodies, 6.9% (n=6) were in Famagusta, 2.7% (n=2) in Kyrenia, 0.01% (n=1) in Nicosia, 4.5% (n=2) in Morphou and 1.2% (n=1) in Trikomo (Table III). Distribution of anti-HEV IgG and IgM were not significantly different according to locations ( $p < 0.05$ ;  $p = 0.101$ ,  $p = 0.462$ , respectively) (Table III).

### Discussion

Cyprus is in the Mediterranean Sea. Until the current study, there has been no surveillance of the seroprevalence and epidemic of HEV infections in Cyprus. The seroprevalence has been changing, and there is a link to geographical locations between 0.0% to 0.9% anti-HEV IgM and 1.1% to 14.2% for anti-HEV IgG depending of the region of Iran (Taherkhani and Farshadpour 2016). Outbreaks of HEV, as such, have not occurred and the prevalence of sporadic cases is lower than 25% (Mohebbi et al. 2012).

In Europe, HEV seroprevalence is predicted to be from 7.5% to 31.9%, with an average of 19.16%. Nonetheless, the real seroprevalence could change due to differences in test sensitivity and the number of asymptomatic courses of the HEV disease (Mauceri 2018). The prevalence rates of anti-HEV in Europe, especially in France (17%) and Germany (35%), could again be based on the pork consumption (Melgaco 2018). There is no published report to allow a comparison with HEV seroprevalence in the Cyprus population.

Prevalence of HEV viremia in blood donors ranges between 1/762 in the Netherlands and 1/9500 people in the United States. Especially immunosuppressed recipients are under the risk of the HEV infection by contaminated blood products (Niederhauser 2018). In Turkey, the total HEV seroprevalence rate was found to be equal to 4.4% (Aydın et al. 2016). In our control group which included blood donors, anti-HEV IgG seropositivity is 1%. This result indicated that anti-HEV seroprevalence was low in our general popula-

tion. According to the previous studies, the exposure to and more specifically devouring of rare meat products are the most important risk factor for HEV transmission (Melgaco 2018). In North Cyprus, the consumption of rare or raw meat is really low which may be a reason that the seroprevalence is lower than in the other countries.

El-Tras et al. (2013) reported 26.8% seropositivity in males, although the female seropositivity was 50.8% in Egypt, and there were significant differences ( $p = 0.05$ ). Altındış et al. (2000) indicated that female seropositivity was 6.7% and male positivity was 3.7% in Turkey. In the current study, anti-HEV IgG antibody concentrations among females were 2.5-fold higher than those in males and this difference was not statistically significant. Our prediction found a higher anti-HEV IgG seroprevalence in female because females could be more involved in activities of supplying animals so that they were more often exposed to animals. Interestingly, anti-HEV IgG seroprevalence was high in male in our study. The majority of the anti-HEV IgG seropositive farmers live in Famagusta. Famagusta has a high proportion of people whose have own farmers. An association to transmission may exists probably through animal wastes and other tissues. Our study has some limitations since statistical studies were difficult due to low overall seroprevalence for HEV in Cyprus.

The prevalence of HEV ranged from 0 to 12.4% in Turkey. The high seroprevalence was detected in agriculture workers (35%). Leblebicioğlu and Ozaras (2018) suggested that fecal route is not a main way of transmission to the HEV in Turkey. Also, low socio-economical status could be related with the seroprevalence of the HEV. The seroprevalence rates were higher for peoples which live in camps or work in agriculture and animal husbandry (Leblebicioğlu and Ozaras 2018). There have been a few studies reported the seroprevalence of anti-HEV-IgG in agricultural workers as being in the range from 4.4% to 34.8%, a 11.5-fold higher than the control groups in Turkey (Ceylan et al. 2003). Eker et al. (2009) reported that 28.5% of seropositive cases were associated with animals such as goats, sheep and cattle. Leblebicioğlu and Ozaras (2018) reported that Turkey is among the countries where HEV is endemic, but there were some limitations to their study since they did not cover the entire population. Aydın et al. (2016) reported that seroprevalence of HEV in animal workers were 35.9% and most frequent in animal husbandry (Aydın et al. 2016). Our results demonstrated a higher anti-HEV IgG seroprevalence among those who spent over 20 years working with animals as compared to less than 20 years. Thus, the time of contact with animals is positively correlated with the risk of infection.

Studies which are designed for the surveillance of HEV antibodies in both animals and humans will

contribute to a better understanding of the seroprevalence of HEV. Close monitoring by the government of precautions when working with animals, high socio-economical status and high educational level may be important to employees in order to decrease the prevalence of the diseases. Currently, there is no data recorded within the Health Ministry of Cyprus regarding HEV infections; thus, the current findings represent the first record of HEV surveillance in Cyprus.

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#### Conflict of interest

Author does not report any financial or personal connections with other persons or organizations, which might negatively affect the contents of this publication and/or claim authorship rights to this publication.

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