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Prevalence and risk factors of Hepatitis E virus infection among Bactrian camel herders in Bayankhongor province, Mongolia

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Abstract

Background and Aim: Hepatitis E virus (HEV) poses a global public health concern. HEV has a single serotype and 8 genotypes. There is inadequate knowledge about risk factors and zoonotic transmission pathways of hepatitis E virus in Mongolia, although the prevalence of HEV is, on average, 12% among the Mongolian population. This study aimed to estimate the prevalence and risk factors of HEV G8 infection in Bayankhongor province, Mongolia.

Materials and Methods: Human serum samples (309) were collected in Bogd, Bayangovi, and Bayanlig districts of Bayankhongor province, Mongolia, with a control group consisting of non-camel herders. An in-house indirect enzyme-linked immunosorbent assay kit was used to detect anti-HEV IgG. The interviewer-administered questionnaire was used to gather the data. The assessment factors included age, sex, and occupation for the respondents. Univariate analyses were conducted using the Statistical Package for the Social Sciences version 26. The same population was tested for hepatitis B and C viruses (HBVs and HCVs) using rapid tests.

Results: Of 309 samples, 41 (13.3%) tested positive for anti-HEV immunoglobulin G antibodies, 23 (7.4%) for HBV and 11 (3.6%) for HCV. HEV seropositivity was linked with water supply, khoormog consumption, and co-infection with HBV and HCV.

Conclusion: HEV prevalence among Bayankhongor residents underscores potential transmission routes related to water supply and khoormog consumption, emphasizing the need for sequencing from human positive sera of HEV and preventive measures. We did not conduct the genotyping of positive human samples.

Keywords: Bactrian camel herder, genotype 8, hepatitis E virus, indirect enzyme-linked immunosorbent assay, seroprevalence.

Introduction

Mongolia faces major health issues, specifically in addressing communicable diseases like viral hepatitis. The world's highest incidence of liver cancer is borne by the country [1–3]. In Asia, Africa, and Central America, hepatitis E virus (HEV) is the major cause of acute hepatitis [4, 5]. HEV has a single serotype and 8 genotypes. The recently identified genotype 8 (G8) of HEV infects camels, including Bactrian camels. Camel herders in rural Mongolia still have uncertainties about HEV infection. Acute human hepatitis worldwide is primarily caused by HEV, a member of the *Hepeviridae* family, subfamily *Orthohepevirinae*, and genus *Paslahepevirus* [6]. HEV, a non-enveloped virus with a single-stranded, positive-sense RNA, infects camels, pigs, wild boars, cows, deer, rabbits, and humans [6, 7]. The HEV virus can be spread by asymptomatic animals in their excrement. To date,

eight genotypes of HEV (HEV1–8) have been identified in *Paslahepevirus balayani* and HEV1–4 have frequently been found in human infection. HEV3 and HEV4 can be isolated from pigs and other mammals and are the major causes of zoonotic hepatitis E [6]. In Japan, HEV5 and HEV6 have been identified in wild boars [8], whereas in camels, HEV7 and HEV8 have been detected [7, 9–11]. A liver transplant patient was chronically infected by HEV genotype 7, which was found in a dromedary camel. Cynomolgus macaques became infected with HEV G8 through exposure to samples that were susceptible to both acute and chronic hepatitis E infection. Further research is necessary to detect G8 occurrences in humans [12]. One hundred twenty-one human and animal HEV sequences from Mongolia, which are deposited in NCBI database, representing four genotypes (HEV1, HEV3, HEV4, and HEV8) and their respective subtypes, were predominant in Mongolia between 2007 and 2023 [6, 7]. A subtype (HEV8a) of the HEV8 virus has been assigned to the Bactrian camel from Mongolia [13]. In Mongolia, the cause of HEV infection among healthy camel herders remains unknown. Consuming undercooked camel meat and raw khoormog in Mongolia might lead to HEV infection. Khoormog, a Central Asian favorite, is a traditional Mongolian beverage made from fermented camel milk that's sparkling white and sour in taste. Monkeys have been shown to be infectable with HEV7 and HEV8, indicating a significant zoonotic risk [14, 15]. The Bactrian camel, native to Central Asia's steppes, is also referred to as the Mongolian camel. 71 of the 200 domestic Bactrian camel serum samples tested from farms in Dornod, Sukhbaatar, and Bayankhongor provinces showed a 35.5% positive rate for anti-HEV immunoglobulin G (IgG); farm (district) prevalence ranged from 4.2% to 75.0%. The highest seroprevalence was recorded in Bogd district, Bayankhongor province [7]. 6-month post-acute HEV infection, anti-HEV immunoglobulin M levels are generally low, whereas anti-HEV IgG remains detectable. In the report of Health indicators-2022 (Ministry of Health, Mongolia), the prevalence of HEV antibodies was low among healthy populations in Ulaanbaatar, Mongolia. The reasons for HEV infections among healthy individuals in Mongolia, based on their residences and habits, remain unclear. The significance of the study was to determine risk factors and transmission pathways of HEV between animals and humans in Mongolia.

The present study aimed to estimate the serological prevalence and risk factors of HEV infections among healthy herders and their family members in Bayankhongor province, Mongolia.

Materials and Methods

Ethical approval and informed consent

The study received ethical approval from the Ministry of Health, Mongolia (Resolution No. 23/02-008) and written consent was obtained from all participants before data collection.

Study period and location

A cross-sectional study was conducted from February 2023 to January 2024 in Bogd, Bayangovi, and Bayanlig districts of Bayankhongor Province.

Data collection

Factors including medical history, livestock contact, consumption of khoormog, and sociodemographic information were gathered through questionnaires for epidemiological analysis. An indirect enzyme-linked immunosorbent assay (ELISA) was used for HEV antibody testing, alongside hepatitis B and C rapid tests.

Serum samples

The prevalence of HEV is, on average, 12% among the Mongolian population. A Cochran's sample size formula and table of Cannon, Roe methods were used to determine the sample size. At 95% with 5% error, the sample should be ≥181. A total of 309 serum samples were collected randomly from adult camel herders (251) and non-camel herders (58) (Figure-1). Samples were stored at ≤−30°C until serological studies were conducted.

ELISA for detecting anti-HEV antibodies

All samples were tested for HEV IgG using the Wantai HEV IgG ELISA kit (Wantai Biological Pharmacy Enterprise Co., Ltd, Beijing, China) at the Mongolian National University of Medical Sciences. Samples were tested according to the manufacturer's instructions, and microplates were washed with a Combiwash workstation (Human Diagnostics, Heidelberg, Germany). Absorbance was measured using a Humareader HS Microplate Reader (Human Diagnostics) at 450 nm. The Wantai HEV IgG assay is based on a recombinant HEV pE2 protein containing 211 amino acids of open reading frame-2 (ORF2) derived from HEV genotype 1 [16, 17]. The sensitivity and specificity of the HEV IgG assay were 97.96% and 99.6%, respectively.

All samples were double tested for HEV IgG with the same HEV ELISA kit for confirmation at the National Center for Communicable Diseases, Mongolia. Absorbance was measured using a Microplate Reader (MPR-D111, MPW-D400 Infitek Inc., Washington, USA) at 450 nm.

Rapid test detection of hepatitis B virus (HBV) and hepatitis C virus (HCV)

HBVs and HCVs were diagnosed using rapid strip tests (Rapigen, Suwon city, Republic of Korea), with sensitivity and specificity ranging from 98.7% to 100.0%. All samples were double tested by rapid strip tests (Safecare Bio-tech, Hangzhou city, China), with sensitivity and specificity ranging from 99.9% to 99.9% respectively.

Statistical analysis

Data analysis was performed using SPSS version 26 (IBM Corp., NY, USA). Univariate analyses were conducted using Fisher's exact test and χ^2 test, with statistically significant differences defined as $p < 0.05$.

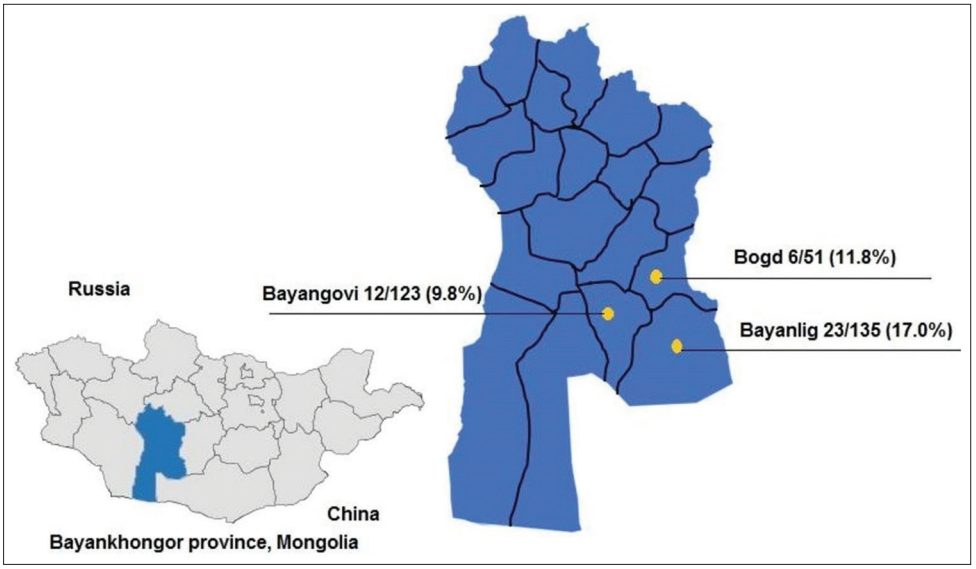

Figure-1: A map of Mongolia showing the locations of the three districts in Bayankhongor province, where serum samples were collected from camel herders and non-camel herders. [Source: The map was generated using ArcGIS 10.4 version (California, United States)].

Adjusted odds ratios and 95% confidence intervals were calculated using univariate logistic regression.

Results

ELISA results for detecting anti-HEV antibodies

Of the samples, 41/309 (13.3%) tested positive for anti-HEV IgG from three districts of Bayankhongor province using both tests (Figure-2). Twelve of 123 (9.8%) in Bayangovi district, 6/51 (11.8%) in Bogd district, and 23/135 (17.0%) in Bayanlig district were positive (Table-1). Positive rates varied across districts, with Bayanlig district exhibited the highest prevalence (17.0%). Twenty-nine out of 251 (11.6%) camel herders were positive, whereas 12/58 (20.7%) non-camel herders were positive. Camel herders showed a lower prevalence (11.6%) compared to non-camel herders (20.7%). Using epidemiological methods, 24/41 (58.5%) were aged between 40 and 59 years. Among those infected by HEV infection, 26/41 (63.4%) were found to have a statistically significant association with the consumption of tank water. Furthermore, although consumption of camel meat did not reach statistical significance, 38/41 (92.7%) cases with HEV infection consumed camel meat. There was no statistical significance in relation to age, sex, location, profession, education, body mass index (BMI), and blood sugar.

Detection of HBV and HCV

Of the samples, 23/309 (7.4%) and 11/309 (3.6%) tested positive for HBVs and HCVs, respectively (Figure-2). Co-infection rates of HEV with HBV and HCV were observed at 1.6% and 0.6%, respectively (Figure-3).

Statistical results

Age, sex, location, profession, education, BMI, or blood sugar were not significantly linked to HEV seropositivity. However, factors such as water supply and accommodation were statistically significant. Those consuming tank water and living in private homes faced a greater risk for hepatitis E infection.

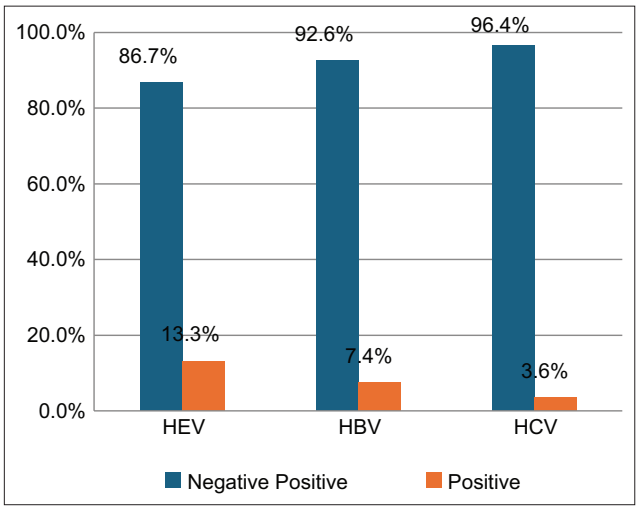

Figure-2: Infection percentages of HEV, HBV, and HCV separately in camel herders of Bayankhongor province, Mongolia. HEV=Hepatitis E virus, HBV=Hepatitis B virus, HCV=Hepatitis C virus.

Discussion

This study aimed to investigate HEV and G8 infection among camel herders by examining their consumption patterns of camelid meat and milk. HEV strains have been identified in camels from Bayankhongor province, Mongolia, where the virus is common and prevalent. Concerns surround HEV G8 due to its zoonotic potential [7]. The prevalence of HEV is, on average, 12% among the Mongolian population. There are 3 zoonotic genotypes, which are G3, G4, and G8 of HEV in Mongolia. G3 and G4 of HEV were found in pigs in Mongolia. G4 of HEV was found in Mongolian sheep. A full-length and three partial HEV genomic sequences of G8 have been

Table-1: Risk factors associated with HEV in camel herders from the Bayankhongor province in Mongolia following a univariable logistic regression analysis.

Variables	Category	Total number	Positive number	Negative number	Positive rate (%)	OR	95% CI	p-value
Age group	40–59	177	24	153	13.6	1	0.43–2.5	0.999
	60+	59	8	51	13.6	1.11	0.39–3.17	0.83
	20–39	73	9	64	12.3	Ref		
Sex	Female	192	27	165	14.0	1.20	0.61–2.46	0.5982
	Male	117	14	103	11.97	Ref		
Location	Bogd	51	6	45	11.8	1.23	0.40–3.46	0.6921
	Bayanlig	135	23	112	17.0	1.90	0.90–4.12	0.088
	Bayangovi	123	12	111	9.8	Ref		
Accommodation	Apartment	14	3	11	21.4	2.12	0.45–7.64	0.2574
	Private house	31	8	23	25.8	2.70	1.05–6.49	0.02316
	Yurt	264	30	234	11.4	Ref		
Water supply	Tank water	18	8	10	44.44	7.28	1.83–32.94	0.001942
	Tap water	16	3	13	18.75	2.16	0.36–11.82	0.335
	Well water	233	26	207	11.16	1.19	0.42–4.2	0.7544
	Spring water	42	4	38	9.52	Ref		
Profession	Non-camel herders	58	12	46	20.7	1.99	0.92–4.2	0.06454
	Camel herders	251	29	222	11.6	Ref		
Education	No education	4	1	3	25.00	3.28	0.09–56.31	0.3382
	High school education	149	18	131	12.08	1.44	0.35–9.78	0.6374
	Middle school education	94	10	84	10.64	1.25	0.28–8.92	0.7831
	Bachelor education	39	10	29	25.64	3.55	0.77–26.02	0.1028
	Primary education	23	2	21	8.70	Ref		
BMI class	Normal	124	20	104	16.13	1.92	0.47–12.99	0.3951
	Overweight	106	13	93	12.26	1.40	0.32–9.75	0.6737
	Obese I	57	6	51	10.53	1.18	0.23–9.06	0.8496
	Obese II	22	2	20	9.09	Ref		
Blood sugar	<5.6	147	22	125	14.97	1.58	0.54–5.67	0.4209
	5.6–6.9	122	15	107	12.30	1.26	0.41–4.67	0.6954
	>6.9	40	4	36	10.00	Ref		
Have you ever had hepatitis A?	Yes	57	7	50	12.28	0.89	0.35–2.08	0.8076
	No	252	34	218	13.49	1.44		Ref
Have you ever been diagnosed with hepatitis?	Yes	82	13	69	15.85	1.34	0.64–2.71	0.4208
	No	227	28	199	12.33	Ref		
Have you ever been treated in a hospital?	No	220	33	187	15.00	1.78	0.81–4.29	0.1585
	Yes	89	8	81	8.99	Ref		
Have you had surgery in the last 6 months?	No	296	40	256	13.51	1.87	0.31–41.41	0.5448
	Yes	13	1	12	7.69	Ref		
Have you had your teeth removed in the past 6 months?	Yes	20	3	17	15.00	1.17	0.26–3.87	0.8134
	No	289	38	251	13.15	Ref		
Have you had an intramuscular injection in the past 6 months?	Yes	86	14	72	16.28	1.41	0.68–2.83	0.3327
	No	223	27	196	12.11	Ref		
Did you get vaccinated against HBV?	Yes	41	4	37	9.76	Ref		
	No	268	37	231	13.81	1.48	0.53–5.13	0.4765
Have you had a blood transfusion for any reason?	Yes	11	0	11	0	Ref		
	No	298	41	257	13.76	Undefined	0.49-Undefined	0.1868
Did you receive folk remedies within the last month?	Yes	25	2	23	8	Ref		
	No	284	39	245	13.73	1.83	0.48–11.9	0.418
Did you get vaccinated in the past 6 months	Yes	39	4	35	10.26	Ref		
	No	270	37	233	13.70	1.39	0.5–4.82	0.553
Did you eat pork in the past 6 months?	Yes	50	9	41	18	1.55	0.66–3.44	0.2827
	No	259	32	227	12.36	Ref		
Do you use camel meat in the food?	Yes	263	38	225	16.8	2.42	0.78–10.27	0.1438
	No	46	3	43	6.52	Ref		
Do you drink khoormog (fermented camel milk)	Yes	241	37	202	16.2	4.9	1.34–31.04	0.03414
	No	68	4	66	2.94	Ref		

CI=Confidence interval, OR=Odds ratio, Ref=Reference, BMI: Body mass index, HEV=Hepatitis E virus, HBV=Hepatitis B virus

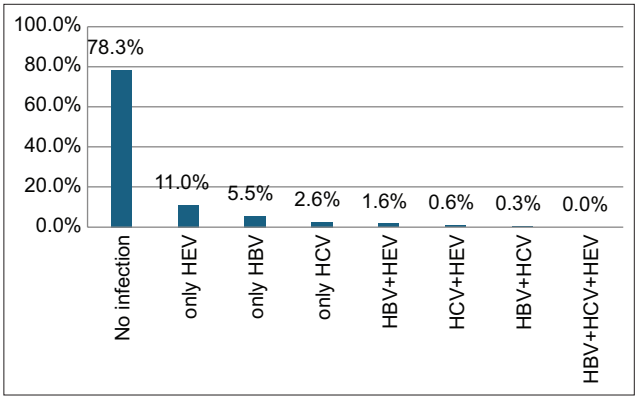

Figure-3: Co-infection percentages of HEV with HBV and HCV in camel herders of Bayankhongor province, Mongolia. HEV=Hepatitis E virus, HBV=Hepatitis B virus, HCV=Hepatitis C virus.

identified from fecal and milk samples of Bactrian camels in a Mongolian south neighboring country, China [9, 10]. The pooled prevalence of anti-HEV IgG in Asia was determined to be 21.03%, with the highest prevalence observed in Myanmar (33.46%) and the lowest in Malaysia (5.93%). IgM prevalence was highest in Indonesia (12.43%) and lowest in Malaysia (0.91%) [18]. In Bayankhongor province, we found a significant number of HEV infections, possibly linked to water supply and khoormog consumption. The association between private housing and HEV transmission might be attributed to potential problems with sewage systems. Non-camel herders, too, showed a significant HEV infection rate, emphasizing the influence of water intake and khoormog consumption on transmission. HEVs can withstand cold and weak acidic conditions like khoormog. These HEVs, being naked and quasi-enveloped, exhibit resistance to alcohols [19]. Most residents in Bayankhongor province, Mongolia, consume tank water and khoormog regardless of daily contact with camels. Based on molecular analysis, HEV and G8 were identified as the causative agents for all confirmed cases, thereby confirming khoormog as the transmission route. Raw camel milk (khoormog) may pose a risk. More research is required to completely elucidate transmission patterns.

Conclusion

13.3% of tested residents of Bayankhongor province have HEV infection, compared to 11% of the national population, highlighting a notable public health issue. Our findings emphasize the importance of preventing HEV transmission, which advocate for improved water sanitation and safe consumption practices related to camelid products. The lack of molecular confirmation for all seropositive cases underscores the importance of additional molecular analysis to definitively identify HEV G8 infections. Future research should prioritize sequencing positive HEV samples and adding them to GenBank for enhanced insight into HEV transmission in Mongolia. Continued surveillance and research are essential to develop comprehensive strategies for HEV prevention and control, ultimately reducing the burden of HEV-related diseases in Mongolia.

Authors' Contributions

EB: Data analysis and data curation and drafted and revised the manuscript. BEC and SG: Laboratory analysis. KN, BO, DO, TD, BB, and TIM: Conceptualization and resources. SOK: Data analysis. ED, OR, OM, EY, and MA: Data curation. BTO: Administration, funding acquisition. formal analysis and investigation. All authors have read, reviewed, and approved the final manuscript.

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Competing Interests

The authors declare that they have no competing interests.

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