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Article:

Study on the incidence of Hepatitis A virus in human and raw milk in Sohag city

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Abstract

Hepatitis A virus (HAV) is the common cause of acute Hepatitis A. It is one of the primary causes of food-borne outbreaks especially consumption of raw milk. This cross-sectional study was conducted to assess the incidence of HAV antibodies (IgM & IgG) in raw milk collected from dairy cows and serum samples of patients and detect the risk factors associated with HAV infection. Raw milk (44) samples and blood serum samples (90) from patients were subjected to ELISA using commercial kits for IgM and IgG antibodies. Variables associated with HAV infection were assessed using bivariate logistic regression analysis. Incidences of HAV-IgM and IgG in raw milk samples were 15.9% (7/44) and 2.3% (1/44), respectively, but both antibodies were not detected together. While incidences of HAV-IgM and IgG in human serum samples were 10% (9/90) and 92.2% (83/90), respectively and both antibodies were detected in 8.9% (8/90) samples. These results suggest that raw milk from infected dairy cows constitute a potential zoonotic risk to humans which pointed out the importance of continuous surveillance to reduce the burden of HAV that represent major public health threat. Statistical analysis showed that previous infection with hepatic diseases (HCV & HBV), blood transfusion, contact with animals, the source of drinking water, the source of food and sewage disposal are risk factors for HAV-IgM infection (*p. value* < *.05*), while gender, presence of pregnancy in females and the source of drinking water are predisposing factor for HAV-IgG infection (*p. value* < *.05*).

Keywords: Hepatitis, HAV, raw milk, serum, ELISA.

Introduction

Foodborne diseases induced by viral pathogens represent important global public health issue, among these viruses Hepatitis A virus [1]. It is an acute, self-limiting enteric foodborne viral zoonosis caused by Hepatitis A virus (HAV) [2]. Recently it has been considered as a re-emerging foodborne major public health threat all over the world [3]. HAV, is a small non-enveloped, single-stranded RNA virus. It is a member of the genus *Hepatovirus*, family *Picornaviridae* [4]. The virus exhibits remarkable stability against medications, disinfectants and environmental conditions like acidic pH, moderate heating, drying and pressure [5,6]. Thus, it is able to survive on human skin, food items, environmental surfaces, soil and sewage [7]. The primary routes of HAV transmission include the fecal-oral route and ingesting contaminated food or water [8]. Another possible mechanisms of transmission include close personal contact and contact with contaminated fomites [9], parenteral transmission via contaminated blood or blood products [10], and needles/paraphernalia sharing among people who inject drugs [9]. The clinical presentation of the disease ranges from asymptomatic, icteric and fulminant hepatitis [11]. The disease is age-dependent that the severity and fatal outcomes are higher in older people [12]. Children frequently remain asymptomatic, while 70% of adults exhibit both nonspecific and specific signs [13]. The incubation period ranged from 15 to 50 days (average 28 days) [14]. The disease typically starts with a pre-jaundice phase that lasts from 5 to 7 days, during which patients usually develop fever, general malaise, vomiting, abdominal cramps, diarrhea and dark urine. [12,14] Then, the jaundice phase starts and lasts for 4–30 days, and chara-

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-cterized by acholia, jaundice and choluria [15]. The disease normally resolves spontaneously and developing lifelong immunity [16]. Milk serves as an important vehicle for the transmission of foodborne and zoonotic viruses [17]. Pathogenic organisms in the milk are derived from the dairy animal itself (excreted directly into milk), human handlers and the environment. Among various milk-borne pathogens, HAV is a common contaminating organism causing outbreaks of infections and is an indicator of unhygienic conditions during the collection, processing, or storage of milk [18,19]. The number of foodborne HAV outbreaks in milk is underestimated, due to the long incubation period and the consumed milk usually has been discarded before the appearance of the clinical symptoms [14].

Materials and methods

Study design

A total of 44 raw milk samples were collected from dairy farms, and individual animals from home rearing throughout Sohag city, and 90 blood samples were collected from suspected male and female patients (n = 45 for each) in hospitals and private laboratories in Sohag city. All milk and serum blood samples were coded to relate the results of ELISA later for data entry and analysis, then transported to the laboratory in ice boxes and stored at −20 °C until testing.

Data collection

A pretested questionnaire was used to document the demographic data, including gender (male and female) and age (years), which was grouped into seven categories: ≤10, 11–20, 21–30, 31–40, 41–50, 51–60, and ≥61, medical history (hepatic diseases "HCV and HBV", pregnancy in females and blood transfusion), the residence either rural or urban) and exposure to infection (contact with animals, source of drinking water; either tap or water pump, source of food (home food or fast food) and sewage disposal (sanitary drainage system or drainage wells). The questions concerned last the clinical symptoms of the disease (fever, jaundice, dark urine, abdominal cramps and diarrhea).

Ethics statement

This study was approved by the "Institutional Review Board" of the Faculty of Medicine in Assiut University, Assiut, Egypt, with IRP local approval number: 04-2023-200828 and all eligible patients provided informed consent.

Serological diagnosis of the samples

To assess HAV incidence, the presence of HAV-IgM and IgG in milk and human serum samples was determined using a commercial HAV ELISA kit, All the procedures were carried out on each of the samples following protocols as kits manufactured by standard reputed companies: SinoGeneClon Biotech Co.,Ltd, China for HAV-IgM and IgG in milk samples, Imbian laboratory diagnostics, Russia for HAV-IgM and Prechek Bio., Inc., USA for HAV-IgG in human serum samples.

Result interpretation

The optical density (OD) of each sample was read at 450 nm with microplate reader. The interpretations were made according to the instructions, cut-off values for categorization as positive or negative were calculated based on the optical density results for the positive and negative control samples included in each kit.

Statistical analysis

A database was created in MS Excel and analyzed using SPSS 26.0 version, using Chi-square test and bivariate logistic regression analysis to assess the significant factors associated with HAV infection. The odds ratio (OR) and its 95% confidence interval (95%CI) were calculated.

Results

Dairy milk samples

HAV-IgM was detected in 15.9% (7/44) milk samples collected from dairy cows; 3 out of 20 (15%) were from the dairy farms while, 4 out of 24 (16.7%) were from individual cases from home rearing. While all the cows examined from the dairy farms were HAV-IgG negative, only one case from home- rearing cows found to be positive, with a total HAV-IgG incidence of 2.3%. While both HAV-IgM and IgG were not detected in any sample **(Table 1).**

Human blood serum

The incidences of HAV-IgM and HAV-IgG were 10% and 92.2%, respectively. While, both HAV-IgM and IgG were detected in 8.9% of the examined patients **(Table 2).** Regarding risk factors associated with HAV infection, the male- to- female ratio was 1:1, higher HAV-IgM incidence was found among males (15.6%) than females (4.4%). While higher HAV-IgG incidence was detected among females (97.8%) than males (86.7%). HAV-IgM not detected in age groups (≤ 10 and 11-20 years), and the incidences in the following groups (21–30, 31–40, 41–50, 51–60, and ≥61) were 6.3, 14.3, 17.6, 11.8, and 6.3%, respectively. While HAV-IgG incidences revealed that all patients in age groups ≤ 10, 11-20, 21-30 and ≥ 61 years were seropositive, it was 88.2% in both age groups 41-50 and 51-60, while the lowest incidence was detected in the age group 31- 40 years **(Table 3).** Based on medical history, 10.4% of HCV infected patients (n= 48) and 11.1% of HBV infected patients (n=36) found to have HAV-IgM. While

Table (1): Incidence of HAV (IgM & IgG) in the examined raw milk samples

Source of samples	No. of examined samples	Positive HAV samples					
		HAV-IgM		HAV-IgG		Both HAV-IgM & IgG	
		No.	%	No.	%	No.	%
Dairy farms	20	3	15	0	0.0	0	0.0
Home -rearing	24	4	16.7	1	4.2	0	0.0
Total	44	7	15.9	1	2.3	0	0.0

Table (2): Incidence of HAV (IgM & IgG) in patients' blood serum

Type of Ab examined	No. of patients	Positive Ab samples	
		No.	%
HAV-IgM	90	9	10
HAV-IgG	90	83	92.2
Both IgM & IgG	90	8	8.9

Table (3): Occurrence of HAV (IgM & IgG) in patients' blood serum according to demographic s

Variables		No. of patients (90)	Positive HAV-IgM				Positive HAV-IgG			
			No. (9)	%	OR (95% CI)	p- value	No. (83)	%	OR (95% CI)	p- value
Gender	**Male**	45	7	15.6	3.961 (0.775-20.233)	.079a	39	86.7	1	.083a
	Female	45	2	4.4	1		44	97.8	6.769 (0.780-58.732)	
Age group (years)	**≤ 10**	8	0	0.0	1.151 (0.768-1.725)	0.287a	8	100	0.919 (0.590-1.430)	0.406a
	11-20	2	0	0.0			2	100		
	21-30	16	1	6.3			16	100		
	31-40	14	2	14.3			11	78.6		
	41-50	17	3	17.6			15	88.2		
	51-60	17	2	11.8			15	88.2		
	≥ 61	16	1	6.3			16	100		

OR: odds ratio; CI: confidence interval; a: Fisher's Exact test.

91.7% of HCV infected patients (n= 44) and 91.7% of HBV infected patients (n=33) found to have HAV-IgG. Out of 45 females, only 7 were pregnant, one pregnant (14.3%) and one (2.6%) non-pregnant woman found to have HAV-IgM. While, all the pregnant women (100%) participated in our study, and 37 (97.4%) of the non- pregnant were HAV-IgG seropositive. The incidence of HAV-IgM in patients who previously received blood was 33.3% (3/9), while in patients who did not receive blood was 7.4% (6/81). While the incidence of HAV-IgG in patients who had previously received blood was 88.9% (8/9), and patients who did not receive blood was 92.6% (75/81) **(Table 4).** The incidence of HAV-IgM among patients living in rural regions was 10.4% (6/58), compared to 9.4% (3/32) in urban regions. While a higher HAV-IgG incidence was recorded among rural people (94.8% obtained from 55 out of 58) than people from urban regions (87.5% obtained from 28 out of 32) **(Table 5).** Patients who were in contact with animals had a higher HAV-IgM incidence (17.1% obtained from 6 out of 35) than those who had no animal contact (5.5% obtained from 3 out of 55). While HAV-IgG represented 94.3% (33/35) in patients in contact with animals and 90.9% (50/55) in patients who had no animal contact. The incidence of HAV-IgM among patients who used water pumps (50%) is higher than those who consumed tap water (8.1%). While all patients who used water pumps were

Table (4): Occurrence of HAV (IgM & IgG) in patients' blood serum according to medical history

Variables		No. of patients (90)	Positive HAV-IgM				Positive HAV-IgG			
			No. (9)	%	OR (95% CI)	p-value	No. (83)	%	OR (95% CI)	p-value
Hepatic diseases	HCV	48	5	10.4	1	.030*a	44	91.7	1	0.648a
	HBV	36	4	11.1	1.075 (0.267- 4.325)		33	91.7	1 (0.209- 4.776)	
Pregnancy	Yes	7	1	14.3	6.167 (0.338 - 112.402)	0.219a	7	100	1.320 (0.935-2.372)	0.002*a
	No	38	1	2.6	1		37	97.4	1	
Blood transfusion	Yes	9	3	33.3	6.250 (1.242-31.463)	0.026*a	8	88.9	1	0.535a
	No	81	6	7.4	1		75	92.6	1.562 (0.167- 14.662)	

OR: odds ratio; CI: confidence interval; a: Fisher's Exact test, *: significant factor (*p. value < .05)*.

Table (5): Occurrence of HAV (IgM & IgG) in patients' blood serum according to the residence

Type of esidence	No. of patients (90)	Positive HAV-IgM				Positive HAV-IgG			
		No. (9)	%	OR (95% CI)	p- value	No. (83)	%	OR (95% CI)	p- value
Rural	58	6	10.4	1.115 (0.259- 4.759)	0.102_a	55	94.8	2.619 (.548-12.521)	0.201_a
Urban	32	3	9.4	1		28	87.5	1	

OR: odds ratio; CI: confidence interval; a: Fisher's Exact test.

positive for HAV-IgG (100%), which is higher than those who used tap water (90.9%). We detected a higher incidence of HAV-IgM in patients who consumed fast food (16.2%) than those who consumed home food (5.7%). On the other hand, HAV-IgG incidences in patients who consumed home food or fast food were close relatives (94.6% and 90.6%), respectively. Patients who live in a compartment with sanitary drainage system represented lower incidence of HAV-IgM (7.1%) than those who live in a compartment with drainage wells (20%). While HAV-IgG incidences were nearly similar in both patients who live in a compartment with sanitary drainage system (92.9%) and those who live in a compartment with drainage wells (90%) **(Table 6).** Among HAV-IgM positive patients, we detected fever in 4.4% (4/90), while jaundice, dark urine, diarrhea and abdominal cramps were detected in 2.2% (2/90) for each. While among HAV-IgG positive patients, we detected fever in 28.9% (26/90), jaundice in 12.2% (11/90), and dark urine in 7.8% (7/90), while diarrhea and abdominal cramps were detected in 22.2% (20/90) **(Table 7).**

Discussion

There has been a significant increase in the occurrence of foodborne outbreaks linked to the use of milk and dairy products that are caused by hepatitis viruses [20]**.** Among various milk-borne pathogens, HAV is a common contaminating organism causing outbreaks of infections and is an indicator of unhygienic conditions during collection, processing, or storage of milk [18,19]**.**, this was confirmed by our results. Several studies agreed with our results **(Table 1)**, which identified HAV in raw milk samples with percentages of 34.48% in Mashhad, Iran [21], 25.81% in Qazvin, Iran [22], 3.25% in Cairo, Egypt [23] and 1.48% in Dakahlia, Egypt [24]. While Terzi *et al*. [19] failed to detect HAV in the milk samples examined in Turkey. The variation in contamination levels of raw milk with HAV may be related to variations in infection between dairy cows due to different rearing systems in different regions as in some countries the main type of farms are mixed farms which facilitate disease transmission between

Table (6): Occurrence of HAV (IgM & IgG) in patients' blood serum according to exposure to infection

Risk factor		**No. of patients (90)**	**Positive HAV-IgM**				**Positive HAV-IgG**			
			No. (9)	**%**	**OR (95% CI)**	**p- value**	**No. (83)**	**%**	**OR (95% CI)**	**p- value**
Contact with animals	Yes	35	6	17.1	3.586 (0.834-15.418)	0.002*a	33	94.3	1.650 (0.302-9.011)	0.440a
	No	55	3	5.5	1		50	90.9	1	
Source of drinking water	Tap water	86	7	8.1	1	0.003*a	79	91.9	1	0.003*a
	Water pumps	4	2	50	11.286 (1.373-92.796)		4	100	1.467 (0.168-9.958)	
Source of food	Home food	53	3	5.7	1	0.023*a	48	90.6	1	0.390a
	Fast food	37	6	16.2	3.226 (0.752-13.842)		35	94.6	1.823 (0.334-9.946)	
Sewage disposal	Sanitary drainage system	70	5	7.1	1	.001**a	65	92.9	1.444 (0.258-8.074)	0.489a
	Drainage wells	20	4	20	3.250 (0.782-13.50)		18	90.0	1	

OR: odds ratio; CI: confidence interval; a: Fisher's Exact test, *: significant factor, **: highly significant factor (p. value < .05).

Table (7): Clinical symptoms appeared on patients examined against HAV (IgM & IgG)

Clinical symptoms		No. of patients (90)	Positive HAV-IgM			Positive HAV-IgG		
			No. (9)	**%**	**p- value**	**No. (83)**	**%**	**p- value**
Fever	Yes	27	4	14.8	0.262_a	26	96.3	0.320_a
	No	63	5	7.9		57	90.5	
Jaundice	Yes	11	2	18.9	0.302_a	11	100	0.388_a
	No	79	7	8.9		72	91.1	
Dark urine	Yes	8	2	25	0.031^*_a	7	87.5	0.491_a
	No	82	7	8.5		76	92.7	
Diarrhea & abdominal cramps	Yes	21	2	9.5	0.329_a	20	95.2	0.481_a
	No	69	7	10.2		63	91.3	

a: Fisher's Exact test, *: statistically significant factor (*p. value < .05*).

different animal species and also different hygienic standards applied in each farm. Additionally, the poor hygienic conditions during milking process as milking in a polluted environment, fecal contamination of hands of the milker or water used to wash the udder and cleaning of milking utensils. Higher incidences of HAV-IgM and IgG in milk samples was detected in home rearing dairy cows than those from the dairy farms which could be linked to poor hygiene during animal rearing in houses which lead to infection of the animals and the poor awareness about isolation of diseased animals to prevent diseases transmission to healthy animals by contact or through feces and urine of infected animals. The incidence of HAV-IgM among examined patients was 10%, this result is nearly similar to that obtained by Diviza *et al*. [25] who detect HAV-IgM in 10.4% of patients, lower incidence was reported by Zakaria *et al*. [26] as 2.1%, on the other hand, Coursaget *et al*. [27] and Zakaria *et al*. [28] recorded higher incidences as 33% and 34%, respectively. The lower HAV incidence could be explained by the significant improvement in the quality of life including higher quality of fresh foods, improvements in water treatment and water supply systems and sewage disposal during last years which participate in decreasing the incidences of some communicable diseases. The incidence for HAV-IgG among the patients was 92.2%. A nearly analogous result was obtained by Kotwal *et al*. [29] and e Araújo *et al*. [30] who reported HAV-IgG incidence of 92.68% and 89.1%, respectively. Lower HAV-IgG incidences was detected by Sıdal et al. [31] as 29%, Assis *et al*. [32] as 86.4%, Sencan *et al*. [33] as 68.8%, Yun *et al*. [34] as 63.80%, Sabir e*t al*. [35] as 33.1%, Esmaeilidooki *et al*. [36] as 17.8%, Joon *et al*. [37] as 19.31%, Gupta *et al*. [38] as 43.5%. While Aksu *et al*. [39] and Divizia *et al*. [25] documented higher HAV-IgG incidences as 94% and 99.5%, receptively. This difference may be attributed to different endemicity of HAV in socio-demographically different study populations. Regarding risk factors associated with HAV infection, higher HAV-IgM incidence was found among males (15.6%) than females (4.4%), this may attributed to that males work outside for longer hours so they are more susceptible for consuming fast food as ingesting contaminated food or water is the main route for transmission of HAV infection [40]. But higher HAV-IgG incidence was detected among females (97.8%) than males (86.7%). This is in agreement with studies who documented higher HAV-IgG seropositivity among females than males [30,34,38]. The highest HAV-IgM incidence was observed in the age group 41-50 years (17.6%). This could be because they are more exposed to contaminated junk food than younger children. Opposite to our study in which HAV-IgM can't be detected in the age group ≤ 10 years, Divizia *et al*. [25] reported the highest incidence in the age group 0-9 years (64.7%). While all patients in age groups ≤ 10, 11-20, 21-30 and ≥ 61years were HAV-IgG seropositive **(Table 3).** These findings may be the consequence of the group effect, as HAV antibodies are created throughout life and infection typically occurs at a young age [41,42]. The superinfection of HAV with other viruses such as HEV, HBV and HCV, may impact the natural course of the primary disease and result in liver failure which aggravate the condition and lead to more serious outcome [43,44]. We found that 10.4% and 11.1% of HCV and HBV infected patients, respectively, have HAV-IgM. While 91.7% of both HCV and HBV infected patients found to have HAV-IgG. Also Zakaria *et al*. [26] could detect dual infections with HAV and HBV in 2% of the study population. We could detect HAV-IgM in only one pregnant and one non-pregnant woman. While all the pregnant women (100%) and 37 (97.4%) of the non- pregnant were HAV-IgG seropositive **(Table 4).** These results indicated that pregnancy considered an important factor in HAV infection. There is a possible transmission of HAV through blood or blood products [9,10]. So, we studied blood transfusion as a possible risk factor for HAV infection and found that 33.3% of patients who previously received blood and 7.4% of patients who did not receive blood were HAV-IgM positive. While the incidence of HAV-IgG in patients who have previously received blood were 88.9%, and patients who did not receive blood is 92.6%. With reference to the residence, HAV incidence was slightly higher among patients living in rural regions (10.4% and 94.8%) than those living in urban regions (9.4% and 87.5%) for IgM and IgG, respectively **(Table 5).** Similar to our results, Arif [45] and e Araújo *et al*. [30] reported higher seropositivity of HAV in rural areas than in urban areas, which proves that people from rural regions are more at risky to acquire HAV infection than urban areas. All these findings are concordant with other studies which mentioned that HAV infection is linked to poor socioeconomic conditions, such as crowded living conditions, inadequate infrastructure, and lack of sanitation systems, as these variables are frequently found in rural residency and are important factors in HAV transmission [46,47]. Regarding contact with animals, patients who were usually in contact with animals had higher HAV-IgM and IgG incidences (17.1% and 94.3%) than those who had no animal contact (5.5% and 90.9%), respectively **(Table 6)** . These results are in agreement with Kotwal *et al*. [29] who proved that the regular close contact with domestic animals are considered a risk factor for HAV infection, which may be due to contamination of human food and water by animal sewage. The source of drinking water was studied as a risk factor for HAV infection as drinking contaminated water considered a major route for transmission of the disease [40]. The incidence of HAV-IgM among patients who used water pumps (50%) is higher than those who consumed tap water (8.1%). Lower result obtained by Divizia *et al*. [25] who recorded that 4.7% of the study participants who positive for HAV-IgM were consuming village tap water. While all patients who used water pumps were HAV-IgG positive (100%), which were higher than those who used tap water (90.9%). This can be attributed to that the drainage wells can contaminate water in the deep layers of the earth if the source of water was pumps or wells. These findings are analogous to Gupta *et al*. [38] who reported significantly lower HAV seropositivity among those children consuming safe drinking water (43.4%) than children consuming unsafe drinking water (47.8%). A recent study carried out by Wu *et al*. [48] documented that between 1988 and 2018, global HAV outbreaks were primarily associated with contaminated food. Higher incidence of HAV-IgM was detected in patients who consume fast food (16.2%) than in those who consume home food (5.7%). While HAV-IgG incidences in patients who consumed home food or fast food were close relative (94.6%) and (90.6%), respectively. These results are agree with Kotwal *et al*. [29] who recorded higher HAV-IgG seropositivity among people who regularly consume food outside the home. These results attributed to that fast food have been widely implicated in HAV foodborne outbreaks because it is not subjected to heat or poorly cooked before consumption [40]. The source of food contamination could be HAV- containing fecal materials [49], or infected food handlers who don't practice proper personal hygiene [50]. Patients who live in a compartment with a sanitary drainage system represented a lower incidence of HAV-IgM (7.1%) than those who live in a compartment with drainage wells (20%). While HAV-IgG incidences were nearly similar in both groups (92.9% and 90%, respectively).

Conclusion:

Our results suggest that raw milk from infected dairy cows constitute a potential zoonotic risk to humans. Statistical analysis showed that gender, previous infection with hepatic diseases (HCV & HBV), presence of pregnancy in females, blood transfusion, contact with animals, source of drinking water, source of food and sewage disposal are predisposing factors for HAV- infection (*p. value < .05*).

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