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INCIDENCE AND CIRCULATION OF HEPATITIS E VIRUS AMONG FARMED RABBITS IN EGYPT

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ABSTRACT

The rabbit hepatitis E (rHEV) as been found in farmed, wild and pet rabbits as well as in human patients suggesting zoonotic transmission. The aim of this study was to evaluate the prevalence of HEV-infection in farmed rabbits from hyperendemic regions. Serum anti-HEV was tested by ELISA. We collected blood samples from 235 rabbits from different governorate in Egypt. HEV RNA was tested using RT-PCR with degenerative primers to ORF2 in serum and feces samples from 235 and 323 farmed rabbits respectively at different governorates in Egypt. All animals were 2-12 months of age. Out of the 235 rabbit serum samples, the total positive rate of anti-HEV IgG was 28/235 (11.9%). Anti-HEV IgG prevalence in rabbits was demonstrated by EIA in serum samples in different governorates 13.20%, 13.6 %, 14.6%, 24.2%, 20.0% in Luxor, Assiut, Fayoum, Menoufia, and Alexandria respectively, and not detected in Sohag and Qena governorates. HEV RNA was detected in serum and fecal samples, only 2 serum samples were positive for HEV RNA 2/235 (0.8 %) at Luxor and Menoufia governorates. Out of the 323 fecal samples, the total positive rate HEV RNA was 26/323 (8.0%). Prevalence of HEV RNA in fecal samples of rabbits varied in different studied governorates from 1.92% to 18.36%, in Qena and Menoufia governorates respectively. HEV prevalence peaked in different age groups at different farms, with majority of infections at age low 6 months. Rabbit HEV infection in Egypt was first documented in our study.

Key words: rHEV, anti-HEV IgG, Egypt, RT-PCR.

INTRODUCTION

Hepatitis E virus (HEV) was first isolated in 1983 from a fecal sample of a volunteer infected with other virus isolate from a previous non-A non-B hepatitis epidemic(Balayan *et al*., 1983).

HEV is the sole member in the genus *Hepevirus* in the family *Hepeviridae* (Worm *et al*., 2002). Infect human and animal beings (wild boars, domestic pigs, deer, rabbits and etc). The HEV are small virions of 27-34 nm, which icosahedral, non-enveloped, spherical particles, with a single capsid protein and a linear, positive-sense RNA genome of approximately 7200 bps with three open reading frames (ORFs), where ORF3 partially overlaps ORF2 (Vasickova *et al*., 2007; Meng , 2010).

HEV contains four recognized genotypes that belong to the *Orthohepevirus* and at

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least two putative new genotypes (Smith *et al*., 2013). HEV-1 genotype has mainly been isolated from sporadic and epidemic human patients in Asia. HEV-2 genotype was first isolated in Mexico but endemic in central Africa. HEV-3 was first isolated to cause sporadic disease in the USA, but has also been recorded to be responsible for acquired HEV cases in Europe, Japan, Australia, Korea, Argentina, and New Zealand. HEV-3 genotype is zoonotic and has also been isolated from domestic and wild swine, deer, rats, mongoose, and rabbits (de la Caridad *et al*., 2013; Smith *et al*., 2014; Liu *et al*., 2019). HEV-4 genotype is also zoonotic and is associated with sporadic cases of HEV in humans and infects wild and domestic swine and reportedly cattle and sheep. HEV-4 genotype has been found in sporadic hepatitis E cases in China, Vietnam, Japan and Taiwan. However, the detection of genetically distinct HEV strains in rats, rabbits and wild boars suggests the existence of further HEV genotypes. A putative HEV-5 genotype was isolated from Japanese wild boar (Sonoda *et al*., 2004; Zhao *et al*., 2009; Kwon *et al*., 2012; Petra *et al*., 2013). In addition, HEV-7 genotype was detected in one person in the United Arab Emirates and was associated with several camel products (Lee *et al*., 2016).

HEV is probably hyperendemic in Egypt and seems to be a frequent infection. In Egypt prevalence of HEV in human is higher than other countries. Seroprevalence of HEV rates were High in rural areas of suburban Cairo and Nile Delta. More studies were conducted in Nile Delta, Egypt for anti-HEV seroprevalence and HEV RNA (Darwish *et al*., 2001) while, in animals beings, HEV seropositivity was resulted in 21.6%, 4.4%, 14%, and 9.4% from studied cows, sheep, buffaloes, and goats, respectively (Shata *et al*., 2012). The infected food animals were convenient to positive HEV humans who may estimate the epidemiological picture of potential zoonotic HEV (El-Tras *et al*., 2013). Anti-HEV antibody in horses were also studied as reservoir hosts in Cairo,Egypt (Saad *et al*., 2007).

HEV strain in rabbits was first discovered in 2009 in China, named rabbit HEV (rHEV) (Zhao *et al*., 2009). The presence of rHEV has been further confirmed by different studies on farmed and wild rabbits in the United States (Cossaboom *et al*., 2011), France (Izopet *et al*., 2012), and Russia (Mohammed *et al*., 2015). rHEV has provisionally been assigned to 3ra subtype within HEV genotype 3, to which rHEV is most closely related according to phylogenetic analysis (Purdy *et al*., 2017). However, the characteristic genomic difference between rabbit strains and the representative strains (swine and human strains) of HEV-3 genotype appears to be quite significant. Investigations conducted in China, USA, France, Russia and other countries demonstrated that rHEV is widely prevalent in various breeds of wildlife, farmed, pet and even laboratory rabbits, suggesting that rabbits are likely another key reservoir of HEV (Wang *et al*., 2016).

MATERIALS AND METHODS

Samples and sample collection

Our study first focused on the farmed rabbit (*Oryctolagus cuniculus*) in the Egypt. In this study 235 and 323 serum and fecal samples respectively were collected from different governorates in Egypt, 53 and 64 (Luxor governorate),

22 and 43 (Assiut governorate), 29 and 33 (sohag governorate), 37 and 52 (Qena governorate), 41 and 46 (Fayoum governorate), 33 and 49 (Menoufia governorate), 20 and 36 (Alexandria governorate) serum and fecal samples respectively. Specimens were collected in February and August 2019. The animals were stocked in wood cages and blood samples were collected through venipuncture of the saphenous vein prior to their reintroduction. Serum and fecal samples from rabbits were carried out in a sterile tube and container with a lid and a spoon. The samples were supplied with the accompanying documents by age, place of collection, date. Samples were obtained from each animal individually.

All the sera were classified and stored at -70°C until tested by an ELISA assay following manufacturer's instruction. The ELISA kit used is designed for the qualitative determination of anti- HEV IgG in serum and plasma samples.

Serological detection

Serum Markers for anti-HEV IgG (Takahashi *et al.,* 2005)

Anti-HEV IgG was detected using third generation Enzyme Immunoassay (EIA) according to the manufacturer's instructions (DIA.PRO, Milano, Italy). Since this set is designed to detecting of human anti-HEV IgG, species-specific conjugate in the formulation instead of using the conjugate of the set of anti-rabbit conjugate (goat antibodies, affinity purified, specific for immunoglobulin IgG, IgA, IgM rabbit horseradish-labeled, Russia) at a dilution of 1: 10,000 in phosphate-buffered saline.

The Cut-off was calculated by addition of 0.350 with mean optical density value of the Negative control (NC) and samples were considered as positive when ratio of the test result of sample (odd 450nm) and the cut-off value was above 1 (or ≤1), according to the manufacturer's instruction. Results are read using EL x 800 universal microplate reader, (Biotek Instruments Inc.). All positive samples were retested in duplicate with the same EIA assay to confirm the initial results.

Molecular detection of HEV by RT-PCR (Jothikumar *et al.,* 2006)

RNA extraction

For the isolation of nucleic acids from fecal samples were prepared to 10-20% Clarified fecal extract. For this propose, samples of feces up to 1.0 ml (0.4-1.0 g) was collected with a sterile spatula and placed into a sterile tube. Then 4.0 ml of phosphate buffer solution was added to form slurry of 10-20%. Fecal vigorously vortexed to form a slurry. The resulting suspension was clarified by centrifugation at 3000 rpm for 30 minutes and the supernatants were then transferred into new sterile tubes and centrifuged at 2000 rpm for 20 minutes, to cleaned them from debris and bacterial cells. The supernatant was collected in sterile tubes and stored at -70 °C.

Amplification of DNA fragments by PCR (Huang *et al.,* 2002)

Detection of HEV RNA was performed in a nested polymerase chain reaction with reverse transcription (RT-PCR) with degenerate primers to the site of the open reading frame 2 (ORF 2) HEV (Table 1).

Detection of serum and fecal HEV RNA by nested RT-PCR was performed using a QIAGEN One-Step RT-PCR kit according to the manufacturer's instructions. The primers were adopted after Huang *et al.* (2002). Briefly, a reaction tube contained 50 μL of the

reaction solutions, including 10 μL of the 5 × QIAGEN One-Step RT-PCR buffer, 2 μL of the dNTP mix (containing 10 mM of each dNTP), 10 μL of the 5 × Q-Solution, 2 μL of the external forward and reverse primer (100 pM μL^{-1}) Table 1, 2 μL of the QIAGEN One-Step RT-PCR enzyme mix, 1 μL of the RNase Out RNA inhibitor (10 U μL−1 ; Gibco BRL, Gaithersburg, MD), 10 μL of the template RNA, and 11 μL of the RNase free water. The thermal cycling conditions included one step of reverse transcription for 30 min at 50°C and an initial PCR activation step for 15 min at 95°C. This was followed by 40 cycles of denaturation for 30 s at 94°C, annealing for 30 s at 50°C, and extension for 1 min 15 s at 72°C, and a final incubation for 10 min at 72°C. A nested PCR was conducted with the following components: 3 μL of the RT-PCR product, 5 μL of the 10×PCR buffer, 5 μL of $MgCl_2$ (25 mg mL−1), 4 μL of the dNTP mix (10 mM of each dNTP), 1 μL of the internal forward and reverse primer (100 pm μL−1) Table 1, 0.5 μL of Takara Ex Taq polymerase (5 U μL−1), and 30.5 μL of the double-distilled H_2O. The thermal cycling conditions for the nested PCR included 5 cycles of denaturation for 30 s at 94°C, annealing for 30 s at 45°C, and extension for 1 min 15 s at 72°C. This was followed by 35 cycles of denaturation for 30 s at 94°C, annealing for 30 s at 53°C, and extension for 1 min 15 s at 72°C, and a final incubation for 7 min at 72°C. Sterile distilled water was used as a negative control. The positive control was the strain of human HEV. Positive and negative controls were included in each run with specific molecular weight markers.

Table 1: The oligonucleotides that used for the amplification of HEV RNA

Sequence	Location	Direction	Position in the genome of HEV *
′5-AAT TAT GCC CAG TAC CGG GTT G-3′	External	Forward	5708–5687
′5-CCC TTA TCC TGC TGA GCA TTC TC-3′	External	Reverse	6414–6395
′5-GTT ATG CTT TGC ATA CAT GGC T-3′	Internal	Forward	5993–5972
′5- AGC CGA CGA AAT CAA TTC TGT C-3′	Internal	Reverse	6319–6298

* Numbering of the nucleotide positions given by the strain HEV Burma (number in the database GenBank M73218)

Electrophoresis agarose gel detection

The PCR-HEV product amplified was detected by 1.5% agarose gel electrophoresis, stained with etidium bromide and observed under an ultraviolet light reaction. The expected product of universal nesting RT-PCR is 348 bp.

Statistical Analysis

Data were analyzed using the SPSS version 16. Qualitative variables are described as numbers and percentages. Chi square or Fisher's exact test is used for comparison between groups; as appropriate. Odds ratios and their 95% confidence intervals were calculated. A p value ≤ 0.05 is considered statistically significant.

RESULTS

Serum samples were collected from 235 different farmed rabbits at different governorates in Egypt , 53(22.6%) serum samples were collected from Luxor governorate, 22(9.4%) from Assiut governorate, 29(12.3%) from Sohag governorate, 37(15.7%) from Qena governorate, 41(17.4%) from Fayoum governorate, 33(14.0%) from Menoufia governorate, 20(8.5%) from Alexandria governorate (Table 2). Out of the 235 rabbit serum samples, the total positive rate of anti-HEV IgG was 28/235 (11.9%) examined by ELISA test. Anti-HEV IgG prevalence in rabbits was demonstrated in different governorates 13.2%, 13.6%, 14.6%, 24.2%, 20.0% in Luxor, Assiut, Fayoum, Menoufia, and Alexandria governorates respectively, and not detected in Sohag and Qena governorates. HEV RNA was detected in serum samples, only 2 serum samples were positive for HEV RNA 2/235 (0.8 %) at Luxor and Menoufia governorates (Table 3& Figure 2).

Table 2: Collection of blood samples among rabbits at different governorates in Egypt.

Variables		Frequency	Percent	*P* value
location	Luxor	53	22.6 %	0.022
	Assiut	22	9.4 %	
	Sohag	29	12.3 %	
	Qena	37	15.7 %	
	Fayoum	41	17.4 %	
	Menoufia	33	14.0 %	
	Alexandria	20	8.5 %	
	Total	235	100.0	

Frequency tabulation test

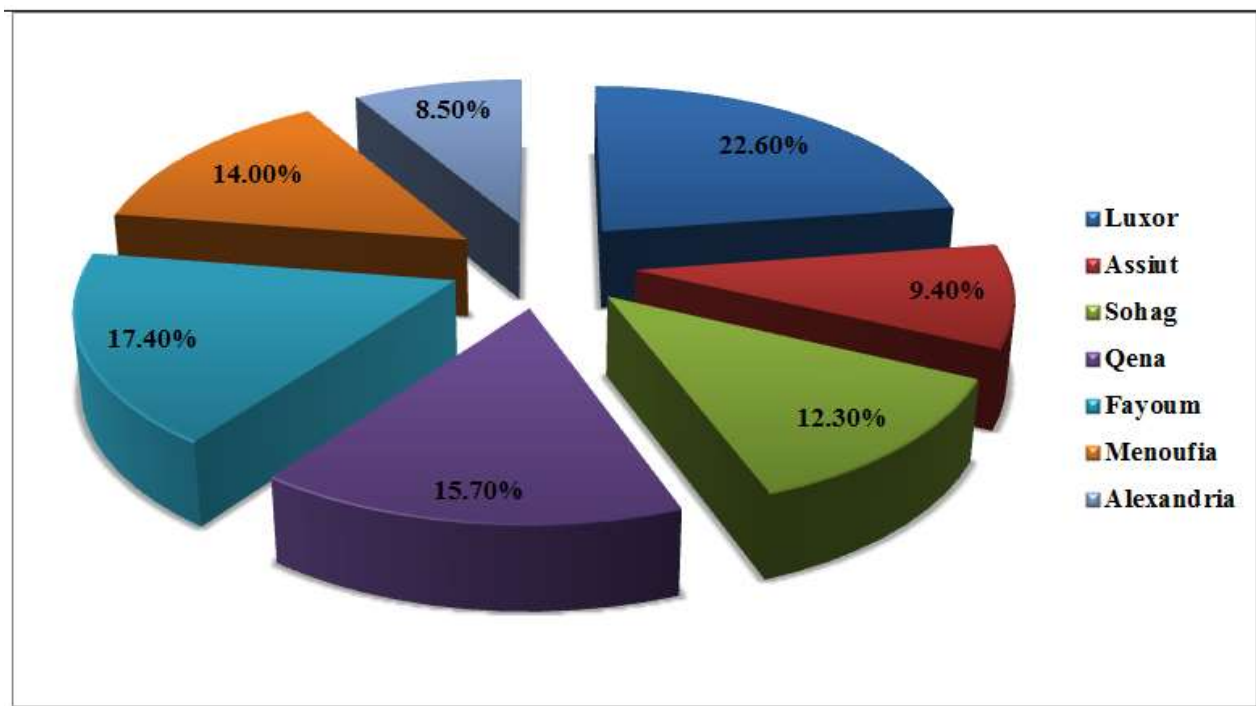

Figure 1: Percentage of collected blood samples among rabbits at different governorates in Egypt

Table 3: Prevalence of HEV markers in serum samples among rabbits at different governorates in Egypt.

Variables			Anti-HEV		HEV RNA	
			Negative	**Positive**	**Negative**	**Positive**
location	Luxor	Count	46	7	52	1
		% within location	86.8%	13.2%	98.1%	1.9%
	Assiut	Count	19	3	22	0
		% within location	86.4%	13.6%	100.0%	0.0%
	Sohag	Count	29	0	29	0
		% within location	100.0%	0.0%	100.0%	0.0%
	Qena	Count	37	0	37	0
		% within location	100.0%	0.0%	100.0%	0.0%
	Fayoum	Count	35	6	41	0
		% within location	85.4%	14.6%	100.0%	0.0%
	Menoufia	Count	25	8	32	1
		% within location	75.8%	24.2%	97.0%	3.0%
	Alexandria	Count	16	4	20	0
		% within location	80.0%	20.0%	100.0%	0.0%
Total		Count	207	28	233	2
		% within location	88.1%	11.9%	99.1%	0.9%
***P* value**			0.017		0.702	
Cross tabulation test						

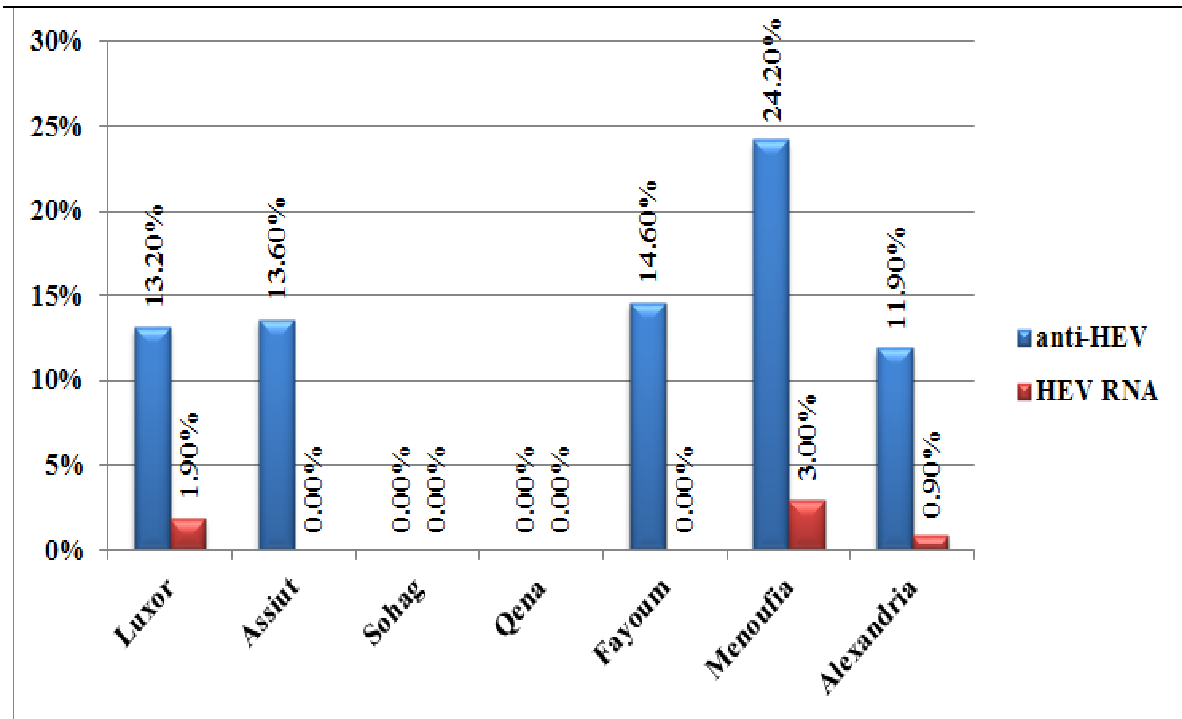

Figure 2: Prevalence of anti-HEV and HEV RNA in serum samples among rabbits at different governorates in Egypt

Fecal samples were collected from 323 different farmed rabbits at different governorates in Egypt, 64 (19.8%) serum samples were collected from Luxor governorate, 43 (13.3%) from Assiut governorate, 33 (10.2%) from Sohag governorate, 52 (16.1%) from Qena governorate, 46 (14.2%) from Fayoum governorate, 49 (15.2%) from Menoufia governorate, 36 (11.1%) from Alexandria governorate (Table 4 & Figure 3). Out of the 323 fecal samples, the total positive rate HEV RNA was 26/323 (8.0%). Prevalence of HEV RNA in fecal samples of rabbits varied in different studied governorates from 1.92% to 18.36%, in Qena and Menoufia governorates respectively. While Prevalence of HEV RNA were recorded 3.1 %, 7.0%, 10.9%, and 18.4% in Luxor, Assiut, Fayoum and Menoufia governorates respectively. HEV RNA in fecal samples was not detectable in sohag governorate (Table 5 & Figure 4).

HEV prevalence peaked in different age groups at different farms, with majority of infections at age low 6 months. HEV RNA in fecal samples at Luxor and Assuit governorates were detected only in fecal samples of rabbits age low 6 months as 5.26 and 16.6 respectively and not detectable in fecal samples of rabbits age high 6 months. Fayoum, Menoufia and Alexandria governorates were recorded high positively of HEV RNA at age low 6 months as 20%, 28% and 31.25 % compared to at age high 6 months 3.84%, 8.33 and 5.0% respectively. However only in Qena governorates HEV infection was detected in rabbit with age high 6 months 1 (3.7%) and not detectable in rabbit with low age 6 months (Table 6 & Figure 5).

Table 4: Collection of fecal samples among rabbits at different governorates in Egypt

Variables		Frequency	Percent	*P* value
location	Luxor	64	19.8 %	0.037
	Assiut	43	13.3 %	
	Sohag	33	10.2 %	
	Qena	52	16.1 %	
	Fayoum	46	14.2 %	
	Menoufia	49	15.2 %	
	Alexandria	36	11.1 %	
	Total	323	100.0	

Frequency tabulation test

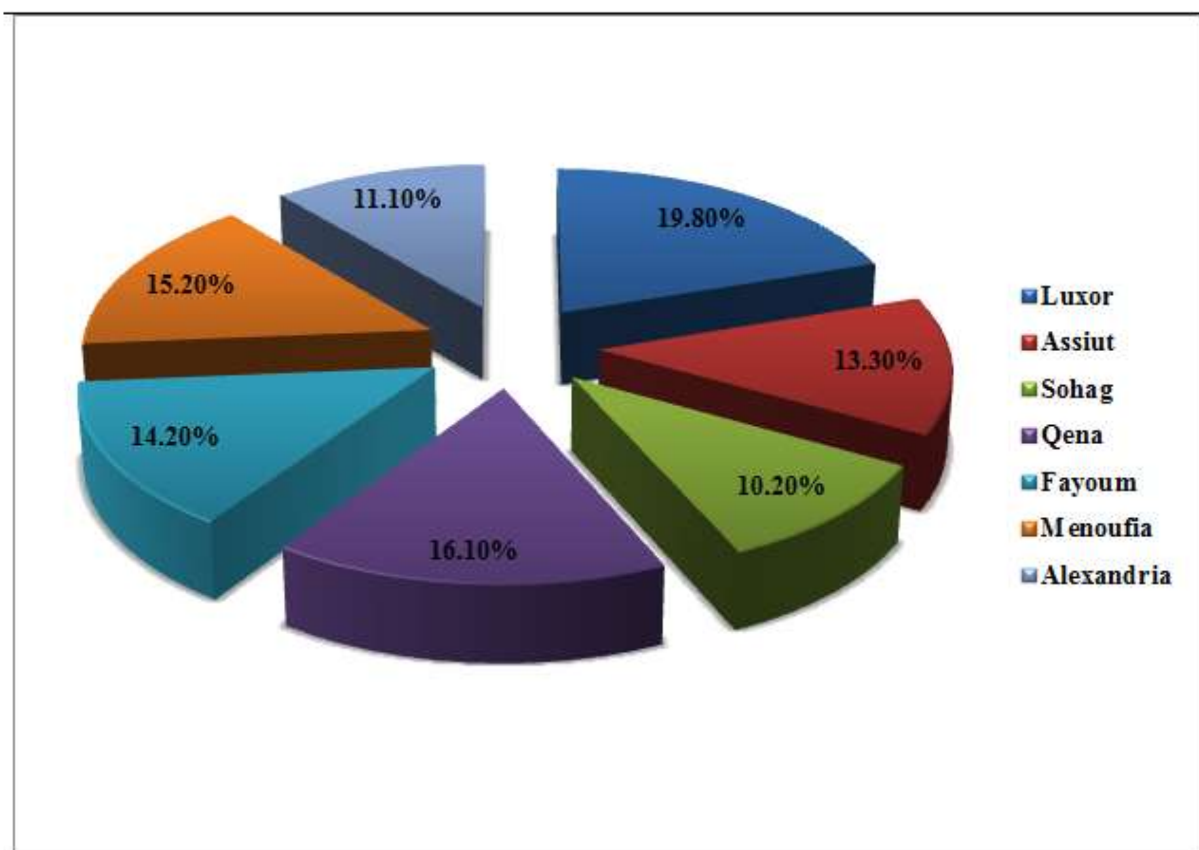

Figure 3: Collection of fecal samples among rabbits at different governorates in Egypt

Table 5: Prevalence of HEV RNA in fecal samples among rabbits at different governorates in Egypt

Variables			**HEV RNA**		**Total**	***P* value**
			Negative	**Positive**		
location	Luxor	Count	62	2	64	0.004
		% within location	96.9%	3.1%	100.0%	
	Assiut	Count	40	3	43	
		% within location	93.0%	7.0%	100.0%	
	Sohag	Count	33	0	33	
		% within location	100.0%	0.0%	100.0%	
	Qena	Count	51	1	52	
		% within location	98.1%	1.9%	100.0%	
	Fayoum	Count	41	5	46	
		% within location	89.1%	10.9%	100.0%	
	Menoufia	Count	40	9	49	
		% within location	81.6%	18.4%	100.0%	
	Alexandria	Count	30	6	36	
		% within location	83.3%	16.7%	100.0%	
Total		Count	297	26	323	
		% within location	92.0%	8.0%	100.0%	

Cross tabulation test

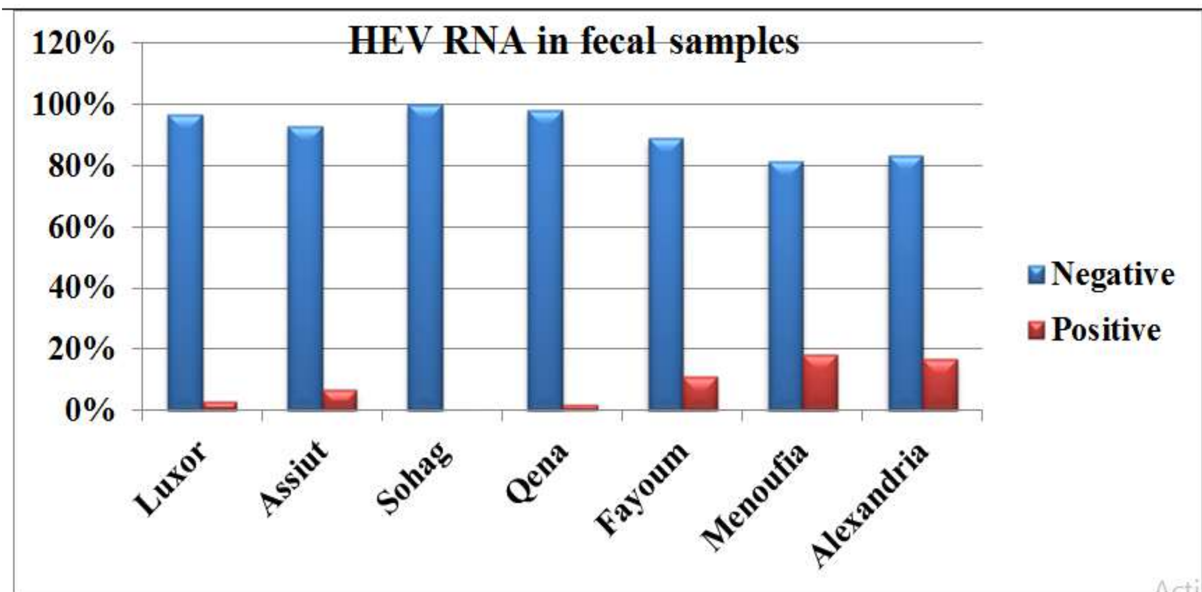

Figure 4: Prevalence of HEV RNA in fecal samples among rabbits at different governorates in Egypt.

Table 6: Age-specific prevalence of HEV infection at different Egypt governorates among rabbits population.

Variables		**Total**	**Ages (months)**	**HEV RNA**		***P* value**
				Total	**Positive**	
location	Luxor	64	≤ **6**	38	2 (5.26%)	0.018
			> **6**	26	0.0	
	Assiut	43	≤ **6**	18	3 (16.6%)	
			> **6**	25	0.0	
	Sohag	33	≤ **6**	22	0.0	
			> **6**	11	0.0	
	Qena	52	≤ **6**	25	0.0	
			> **6**	27	1 (3.7%)	
	Fayoum	46	≤ **6**	20	4 (20%)	
			> **6**	26	1 (3.84%)	
	Menoufia	49	≤ **6**	25	7 (28%)	
			> **6**	24	2 (8.33%)	
	Alexandria	36	≤ **6**	16	5 (31.25%)	
			> **6**	20	1 (5.0%)	
Total				323	8.0 %	

Cross tabulation test

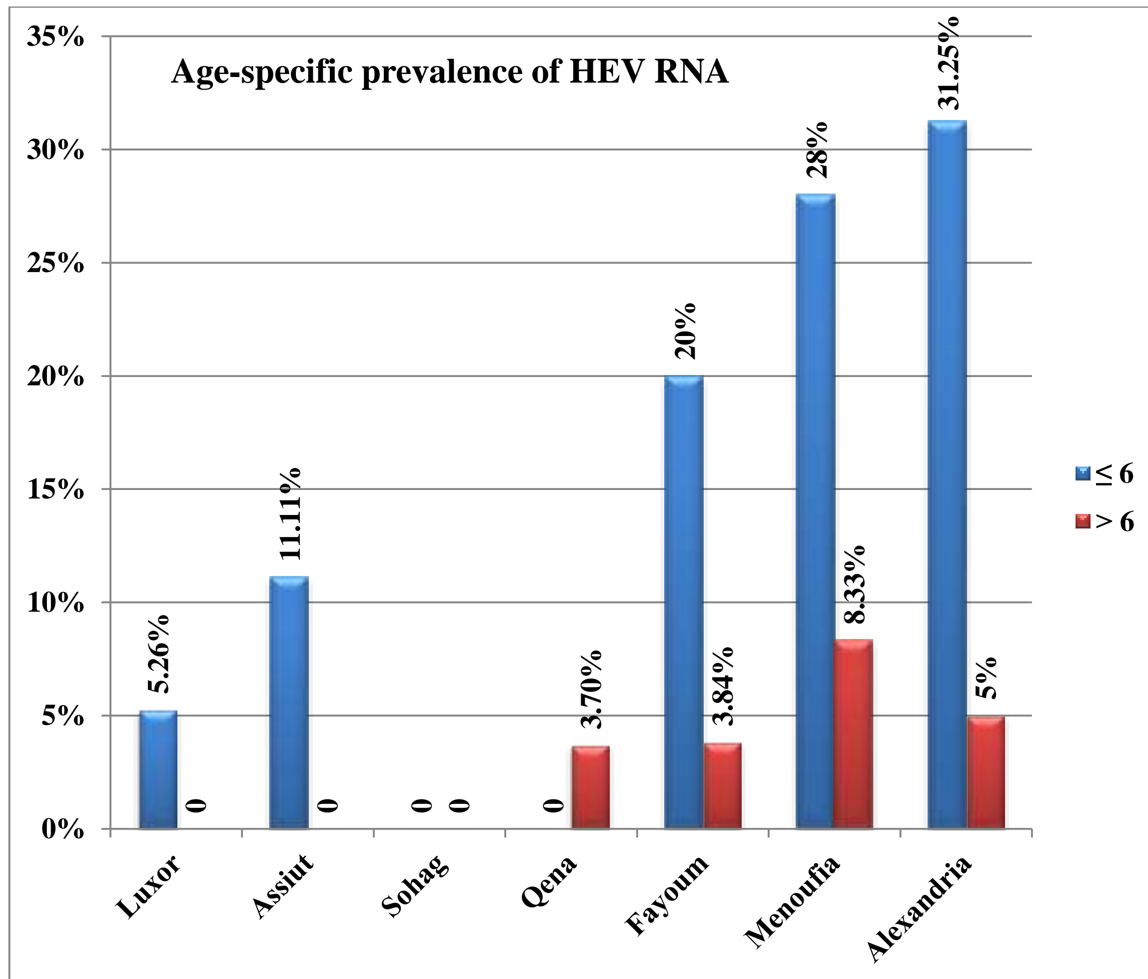

Figure 5. Age-specific prevalence of HEV infection at different Egypt governorates among rabbits population.

DISCUSSION

rHEV was allowed to infect pigs and monkeys through experimental inoculation. However, investigations conducted separately in several areas in China where rHEV and HEVgenotype 4 were highly prevalent in farmed rabbits and pigs respectively, could not observe cross-species infection of HEV strains among rabbits and swine (Liu *et al.*, 2019). In these regions, all of the HEV strains detected from hepatitis E patients belong to HEV genotype 4 of which the nucleotide sequence was highly similar to swine HEV genotype 4 sequences indicating the transmission risks of pigs, but not rabbits (Faber *et al.*, 2018). Only a few strains detected from patients with HEV infection in France have been identified to have similar sequences with the rHEV (Abravanel *et al.*, 2017). Thus far, the contribution of rHEV to human infection remains uncertain.

Present study was recorded out of 235 different serum samples from farmed rabbits at different governorates in Egypt , the total positive rate of anti-HEV IgG was 28/235 (11.9%), and HEV RNA was 2/235 (0.8 %). Agreement with previous studied in France, anti-HEV has been detected in the farmed rabbits, with a positive rate at 7.0%, lower than the wild rabbits at 23.0%. (Izopet *et al*., 2012). However, the prevalence of anti-HEV antibody among rabbits is high in USA, with 36.5% (31/85) positive rate and the detection of rHEV RNA in serum (16.5%, 14/85) samples indicates widespread rHEV circulation among local rabbit population (Cossaboom *et al*., 2011). In other study, Anti-HEV and HEV RNA were detected in the serum samples of farmed rabbits, and the positive rates were 57.0% (191/335) and 6.9% (23/335), respectively (Zhao *et al*., 2009). Low anti-HEV antibody seroprevlance were found in Italy as 3.40% in 206 farmed rabbits, suggesting that HEV was circulating among rabbits in Italy (Di Bartolo *et al*., 2016). A retrospective study in Germany tested 13 serum samples of wild rabbits, 4/13 (31%) samples were positive for anti-HEV antibodies (Eiden *et al*., 2016).

Anti-HEV IgG prevalence in rabbits was demonstrated in present study at different governorates 13.20%, 13.6%, 14.6%, 24.2%, 20.0% in Luxor, Assiut, Fayoum, Menoufia, and Alexandria governorates respectively, and not detected in Sohag and Qena governorates. HEV RNA was detected in serum samples, only 2 serum samples were positive for HEV RNA at Luxor and Menoufia governorates. However, In China, rabbits in Inner Mongolia have the highest prevalence of anti-HEV IgG (57%) and viraemia (72%). In the US recorded, rabbits in Farm A had a higher prevalence of HEV RNA in serum and fecal samples (48.0% and 40%) compared as in farm B (3.3% and 5.0%). The rabbits in Inner Mongolia were caged in groups of 2 to 336 but 2 to 9 in Farm A in Virginia (Cossaboom *et al*., 2011).

Out of the 323 fecal samples in our study, the total positive rate HEV RNA was 26/323 (8.0%). Prevalence of HEV RNA in fecal samples of rabbits varied in different studied governorates from 1.92% to 18.36%, in Qena and Menoufia governorates respectively. While Prevalence of HEV RNA were recorded 3.1 %, 7.0%, 10.9%, and 18.4% in Luxor, Assiut, Fayoum and Menoufia governorates respectively. HEV RNA in fecal samples was not detectable in sohag governorate. Howere, other studies showed low prevalence rate of fecal HEV RNA at 1.0% involving 3 regions of China, and all rabbits were caged individually, which, in a way reduced the possibility of fecal oral transmission between cage mates (Xia *et al.*, 2015).

Soon afterwards, another study reported a different strain of rHEV in the fecal samples of Rex Rabbits in Beijing. The detection rates of anti-

HEV and fecal HEV RNA were 54.62% (65/119) and 6.96% (8/115), respectively. The detection of HEV RNA in fecal samples indicated the possibility of fecal-oral transmission of rHEV from rabbit to rabbit or to other species of animals (Geng *et al*., 2011). In a recent study, which involved 111 farmed rabbits in Beijing were tested for fecal RNA with a positive rate of 4.6% (5/111). Fecal samples of 285 farmed rabbits in Shandong province and of 96 farmed rabbits in Henan province were also tested, but no HEV RNA was detected. Researchers from the Korea, Netherlands, and Canada have reported detecting HEV RNA in the local rabbit beings, with prevalence ranging from 0.9% to 60% (Xia *et al.*, 2015). In our study, HEV prevalence peaked in different age groups at different farms, with majority of infections at age low 6 months. HEV RNA in fecal samples at Luxor and Assuit governorates were detected only in fecal samples of rabbits age low 6 months as 5.26% and 16.6 % respectively and not detectable in fecal samples of rabbits age high 6 months. Fayoum, Menoufia and Alexandria governorates were recorded high positively of HEV RNA at age low 6 months as 20%, 28% and 31.25 % compared to at age high 6 months 3.84%, 8.33 and 5.0% respectively. Agreement with previous studies have shown that high prevalence of HEV infection at ages of the rabbits less than 3 months for farmed rabbits compared as over 6 months (Mohammed *et al*., 2015; Leblanc *et al*., 2010; Geng *et al*., 2019). Although the prevalence of HEV in farmed rabbit could be linked to their older age. In Qena governorates HEV infection was detected in rabbit with age high 6 months 1 (3.7%) and not detectable in rabbit with low age 6 months.

CONCLUSIONS

Hepatitis E virus is widespread among rabbits in hyperendemic region (Egypt); at this point high frequency of detection of anti-HEV class IgG (11.9%) and HEV RNA (0.8% and 8.0% in blood and fecal samples from farmed rabbits respectively). HEV prevalence peaked in different age groups at different farms, with majority of infections at age low 6 months.

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Conflict of interest statement

None of the authors has an affiliation or conflict of interest.

REFERENCES

Abravanel, F.; Lhomme, S.; El Costa, H.; Schvartz, B.; Peronm J.M.; Kamar, N. and Izopet, J. (2017): Rabbit hepatitis E virus infections in humans, France. Emerging Infectious Diseases. 23: 1191–1193.

Balayan, M.S.; Andjaparidze, A.G.; Savinskaya, S.S.; Ketiladze, E.S.; Braginsky, D.N.; Savinov, A.P. and Poleschuk, V.F. (1983): Evidence for a virus in Non-A, Non-B hepatitis transmitted via faecal-oral route. Intervirology. 20: 23-31.

Cossaboom, C.M.; Cordoba, L.; Dryman, B.A. and Meng, X.J. (2011): Hepatitis E virus in rabbits, Virginia, USA. Emerg Infect Dis. 17:2047-2049.

Darwish, M.A.; Faris, R.; Darwish, N.; Shouman, A.; Gadallah, M.; El-Sharkawy, M.S.; Edelman, R.; Grumbach, K.; Rao, M.R. and Clemens, J.D. (2001): Hepatitis c and cirrhotic liver disease in the Nile delta of Egypt: a community-based study. Am J Trop Med Hyg. 64: 147-53.

de la Caridad Montalvo Villalba, M.; Owot, J.C.; Corrreia, B.; Corredor, M.B.; Flaquet, P.P.; Frometa, S.S.; Wong, M.S. and Rodriguez Lay, L.D. (2013): Hepatitis E virus genotype 3 in humans and swine, Cuba. Infect. Genet. Evol. 14:335–339.

Di Bartolo, I.; De Sabato, L. and Marata, A. (2016): Serological survey of hepatitis E virus infection in farmed and pet rabbits in Italy. Arch Virol. 161:1343-1346.

Eiden, M.; Vina-Rodriguez, A.; Schlosser, J.; Schirrmeier, H. and Groschup, MH. (2016): Detection of hepatitis E virus in archived rabbit serum samples, Germany 1989. Food Environ Virol. 8:105-107.

El-Tras, W.F.; Tayel, A.A. and El-Kady, N.N. (2013): Seroprevalence of hepatitis E virus in humans and geographically matched food animals in Egypt. Zoonoses Public Health. 60: 244–251.

Faber, M.; Willrich, N.; Schemmerer, M.; Rauh, C.; Kuhnert, R.; Stark, K. and Wenzel, J.J. (2018): Hepatitis E virus seroprevalence, seroincidence and seroreversion in the German adult population. Journal of Viral Hepatitis. 25: 752–758.

Geng, J.; Wang, L. and Wang, X. (2011): Study on prevalence and genotype of hepatitis E virus isolated from rex rabbits in Beijing. China J Viral Hepat. 18:661-667.

Geng, Y.; Zhao, C. and Geng, K. (2019): High seroprevalence of hepatitis E virus in rabbit slaughterhouse workers. Transbound Emerg Dis. 66:1085–1089.

Huang, F.F.; Haqshenas, G.; Guenette, D.K.; Halbur, P.G.; Schommer, S.K.; Pierson, F.W.; Toth, T.E. and Meng, X.J. (2002): Detection by reverse transcription-PCR and genetic characterization of field isolates of swine hepatitis E virus from pigs in different geographic regions of the United States. J Clin Microbiol. 40: 1326–1332.

Izopet, J.; Dubois, M. and Bertagnoli, S. (2012): Hepatitis E virus strains in rabbits and evidence of a closely related strain in humans, France. Emerg Infect Dis. 18:1274-1281.

Kwon, H.; Sung, H. and Meng, X.J. (2012): Serological prevalence, genetic identification, and characterization of the first strains of avian hepatitis E virus from chickens in Korea. Virus Genes. 45: 237–245.

Leblanc, D.; Poitras, E.; Gagne, M.J.; Ward, P. and Houde, A. (2010): Hepatitis E virus load in swine organs and tissues at slaughterhouse determined by real-time RT-PCR. Int J Food Microbiol 139:206–9.

Lee, G.H.; Tan, B.H.; Teo, E.C.; Lim, S.G.; Dan, Y.Y.; Wee, A.; Aw, P.P.; Zhu, Y.; Hibberd, M.L. and Tan, C.K. (2016): Chronic infection with camelid Hepatitis E virus in a liver transplant recipient who regularly consumes camel meat and milk Gastroenterol. 150: 355-357.

Liu, B.; Chen, Y.; Sun, Y.; Nan, Y.; Li, H. and Du, T. (2019): Experimental infection of rabbit with swine-derived hepatitis E virus genotype 4. Vet Microbiol. 229:168–75.

Meng, X.J. (2010): Recent advances in hepatitis E virus. J Viral Hepat. 17:153–61.

Mohammed, A.M.E.; Potemkin, I.A.; Carlsen, A.A.; Isaeva, O.V.;

Jothikumar, N.; Cromeans, T.L.; Robertson, B.H.; Meng, X. and Hill, V.R. (2006): A broadly reactive one-step real-time RT-PCR assay for rapid and sensitive detection of hepatitis E virus. J Virol Methods 131:65–71.

Kyuregyan, K.K.; Kozlov, V.G.; Lark, S.V. and Mikhailov, M.I. (2015): The circulation of the hepatitis E virus in rabbits in areas with different degree of endemicity for hepatitis E // Modern problems of science and education. URL:http://www.science-education.ru/12218395.

Petra, L.R.; Ivan, T. and Andrej, K. (2013): Detection of hepatitis e virus in faeces and liver of pigs collected at two Slovenian slaughter houses. Mac Vet Rev. 36: 97–100.

Purdy, M.A.; Harrison, T.J.; Jameel, S.; Meng, X.J.; Okamoto, H. and derVan Poel, W.H. (2017): ICTV virus taxonomy profile: Hepeviridae. Journal of General Virology. 98: 2645– 2646.

Saad, M.D.; Hussein, H.A.; Bashandy, M.M. and Kamel, H.H. and Earhart, K.C. (2007): Hepatitis E virus infection in work horses in Egypt. Infect Genet Evol. 2007 7: 368–373.

Shata, M.T.; Daef, E.A.; Zaki, M.E.; Abdelwahab, S.F.; Marzuuk, N.M.; Sobhy, M.; Rafaat, M.; Abdelbaki, L.; Nafeh, M.A.; Hashem, M.; El-Kamary, S.S.; Shardell, M.D.; Mikhail, N.N.;

Strickland, G.T. and Sherman, K.E. (2012): Protective role of humoral immune responses during an outbreak of hepatitis E in Egypt.Trans R Soc Trop Med Hyg. 106: 613-8.

Smith, D.B.; Purdy, M.A. and Simmonds, P. (2013): Genetic variability and the classification of hepatitis e virus. Journal of Virology. 87: 4161–4169.

Smith, D.B.; Simmonds, P.; Jameel, S.; Emerson, S.U.; Harrison, T.J.; Meng, X.J.; Okamoto, H.; Van der Poel, W.H.M. and Purdy, M.A. (2014): Consensus proposals for classification of the family Hepeviridae. Journal of General Virology. 95:2223–2232

Sonoda, H.; Abe, M.; Sugimoto, T.; Sato, Y.; Bando, M.; Fukui, E.; Mizuo, H.; Takahashi, M.; Nishizawa, T. and Okamoto, H. (2004): Prevalence of hepatitis E virus (HEV) Infection in wild boars and deer and genetic identification of a genotype 3 HEV from a boar in Japan. J Clin Microbiol. 42:5371–5374.

Takahashi, M.; Kusakai, S.; Mizuo, H.; Suzuki, K.; Fujimura, K.; Masuko, K.; Sugai, Y.; Aikawa, T.; Nishizawa, T. and Okamoto, H. (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 (1), 49–56.

Vasickova, P.; Psikal, I.; Kralik, P.; Widen, F.; Hubalek, Z. and Pavlik, I. (2007): Hepatitis E virus: a review. Vet. Med. 52: 365-84.

Wang, L.; Zhang, Y.; Gong, W.; Song, W.T. and Wang, L. (2016): Hepatitis E virus in 3 types of laboratory animals, China, 2012–2015. Emerging Infectious Diseases. 22: 2157–2159.

Worm, H.C.; van der Poel, W.H.M. and Brandst-tter, G. (2002): Hepatitis E: an overview. Microbes Infect. 4: 657-66.

Xia, J.; Zeng, H. and Liu, L. (2015): Swine and rabbits are the main reservoirs of hepatitis E virus in China: detection of HEV RNA in feces of farmed and wild animals. Arch Virol. 160:2791-2798.

Zhao, C.; Ma, Z.; Harrison, T.J.; Feng, R. and Zhang, C. (2009): A novel genotype of hepatitis E virus prevalent among farmed rabbits in China. J Med Virol. 81: 1371–1379.

الإصابة وانتشار فيروس التهاب الكبد E بين الأرانب المستزرعة فى مصر

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يصيب فيروس التهاب الكبد (E) الانسان والعديد من الأنواع الحيوانية. تم عزل HEV في الارانب المستزرعة والبرية والحيوانات الأليفة وسميت العزلات المعزلة (rHEV) وكانت نفس العزلات المعزولة من المرضى من البشر مما يشير إلى انتقال الأمراض الحيوانية للانسان . كان الهدف من هذه الدراسة هو تقييم مدى انتشار عدوى فيروس الالتهاب الكبد من النوع E في الارانب المستزرعة من مناطق الإصابة بالوباء.تم تجميع عدد 235 عينة دم من ارانب مختلفة من محافظات مختلفة بجمهورية مصر العربية وتم فصل البلازما وعمل تحليل الاجسام المضادة بها Anti-HEV IgG كما تم عمل اختبار تفاعل البلمرة المتسلسل وذلك لمعرفة الحمض النووي للفيروس وذلك باختبار HEV RNA لـ ORF2في عينات المصل والبراز من 235 و 323 من الأرانب المستزرعة على التوالي في محافظات مختلفة في مصر. تراوحت اعمار جميع الارانب بين 2-12 شهراً من أصل 235 عينة مصل أرانب ، كان المعدل الإيجابي الإجمالي للاجسام المضادة للفيروس Anti-HEV IgG (28/235) 11.9% كما تم الكشف عن معدل انتشار الاجسام المضادة للفيروس في الأرانب في عينات المصل في محافظات مصر المختلفة وكانت النتائج كالاتي : الأقصر 13.20% ، أسيوط 13.6% ، الفيوم 14.6% ، المنوفية 24.2% ، والإسكندرية % 20.0 ، ولم يتم الكشف عنها في محافظات سوهاج وقنا . تم الكشف الحمض النووي الريبي لفيروس التهاب الكبد E في عينات المصل والبراز للأرانب ، وسجلت النتائج فقط 2 عينة مصل كانت إيجابية لفيروس الحمض النووي الريبي 235/2 (0.8%) في محافظتي الأقصر والمنوفية. وكان من بين 323 عينة براز ، ان المعدل الإيجابي الكلي ل HEV RNA (8.0 26/323 ٪ (وتفاوت معدل انتشار الحمض النووي الريبي لفيروس التهاب الكبد (HEV RNA) في عينات البراز للأرانب في المحافظات محل الدراسة المختلفة من 1.92% إلى 18.36% في محافظتي قنا والمنوفية على التوالي. بلغ انتشار فيروس HEV ذروته في مختلف الفئات العمرية في المزارع المختلفة ، مع وجود غالبية الإصابات في الارانب تحت عمر 6 أشهر. تم توثيق عدوى الأرانب بواسطة فيروس الالتهاب الكبدي من النوع E في مصر لأول مرة في دراستنا. البيانات عن مدى انتشار والتنوع الوراثي لفصيلة الأرانب HEV في المناطق الموبوءة وغير المستوطنة محدودة ، بالإضافة إلى معلومات حول أهمية HEVللأرانب في علم الأمراض البشرية.