DOI https://doi.org/10.5281/zenodo.10049652#283

TWIST, 2024, Vol. 19, Issue 3, pp. 722-727

TWIST

Journal homepage: www.twistjournal.net

Seroprevalence of Human and Canine Hepatitis B Virus in Samarra Province, Iraq

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Abstract
The most extensively researched hepatitis virus, hepatitis-B virus (HBV), is a major cause of liver disease in mammals, particularly in humans. In the current study, enzyme-linked immunosorbent assays were used to (i) estimate levels of antibodies against HBV and (ii) measure the activity of liver function enzymes using serum samples from 50 patients and 78 dogs. The findings demonstrated that the two tests' values were significant in both groups (patients and dogs), but that the dogs test's values were higher than those of the human tests. It is crucial to confirm this evidence using molecular tests because there is significant evidence of canine hepatitis B in the Samarra Province of Iraq.

Keywords
Canine hepatitis, Hepatitis B, Samarra

INTRODUCTION

Hepatitis-B virus (HBV), the most thoroughly studied hepadnavirus, is a major contributor to liver disease in humans. Hepadnaviridae is a family of small, hepatotropic DNA viruses that infect mammals (Magnius et al., 2020). It is unknown if a pathogenic hepadnavirus affects domestic dogs. In Brazil, 10% of sera from domestic wild dogs had HBV DNA present, and a qPCR was used to detect HBV DNA in 6.3% of the sera from dogs in Italy undergoing routine laboratory testing (Vieira et al., 2019; Diakoudi et al., 2022). Further research is necessary to determine whether dogs are susceptible to hepadnavirus infection, not least because they have a high risk of contracting diseases linked to the virus (Choi, Y.R. et al., 2022).

Dogs are typically not kept as pets in Iraq for a variety of cultural and religious reasons. But even in urban areas, stray dogs are a common sight. Investigation of HBV in dogs is crucial in this scenario because dogs may be infected with HBV or an HBV-like virus and there may have been cross-species transmission of HBV among different hosts (Al-Jumaa ZM, Ajaj EA, 2020; Vieira et al., 2022). Preliminary data seem to suggest that dogs can host a wide range of hepadnaviruses, despite the fact that epidemiological studies are still in their infancy. It is still unknown, though, if these viruses are typical of domestic dogs or if they were accidently discovered (Fruci, P. et al., 2023). So, the aim of the current study to investigate the seroprevalence human and Canine hepatitis B virus in Samarra Province, Iraq

MATERIALS AND METHODS

Sample Collection

In this study, 78 different dog breeds from various areas in Samarra Province, Iraq, as well as 50 patients of both sexes, aged 55 to 70, were used. Dogs included with a range of ages (mean=2.5 years, min=1.5 months, and max=5 years). Between April 2022 and April 2023, serum samples were gathered.

Seroanalysis of HBV

In order to determine the levels of antibodies against HBV serum samples from study patients and dogs were collected. The SunLong Biotech Co., Ltd., China, canine HbsAg ELISA kit was used to calculate the levels of anti-HBV antibodies. All quality assurance practices were taken into account. For instance, to avoid cross-contamination, the kit reagents were kept at room temperature, the automated microplate reader was calibrated before the assay, and a fresh pipette tip was used for each sample. Additionally, the ELISA procedure was carried out in accordance with the manufacturer's guidelines. In brief, (i) the average optical density (OD) for the positive control was set at 1.00 and for the negative control at 0.10; (ii) the cutoff value was set at 0.2635 U/L and was calculated as follows in accordance with the manufacturer's instructions: Cutoff value is equal to the average value of a negative control plus 0.15; (iii) serum was classified as canine HbsAg positive if the OD value was greater than the cutoff value and negative if it was less than the cutoff value. Finally, the OD was assessed at a 450 nm wavelength.

Statistical Analysis

Data were summarized, analyses and presented using statistical package for social sciences (SPSS) version 23 and Microsoft Office Excel 2010. Quantitative variables were expressed as mean, standard deviation (SD); whereas, categorical variables were expressed as number and percentage. Chi-square test was used to study association between any two categorical variables. The level of significance was set at $P \leq 0.05$.

RESULTS

The data represent 50 patients in order to assess whether they have viral hepatitis B or not. Only 9 patients out of a total of 50 tested positive for Hepatitis B viruses, while only 8 (10%) out of 78 dogs tested positive for the virus, which is considered to be a high ratio of virus infection.

Table 1 indicates that the mean infected is (0.30214), with (0.622862) standard deviation. Also, the data is normally distributed by the values of (Skewness = 1.827 & Kurtosis = 1.595) which should be in the range ($\pm$1.96).

Table 1 Descriptive Statistics

Statistics		
SMP		
N	Valid	50
	Missing	0
Mean		.30214
Std. Deviation		.622862
Skewness		1.827
Std. Error of Skewness		.337
Kurtosis		1.594
Std. Error of Kurtosis		.662

*If it is (SMP >1), this indicates that the patient has viral hepatitis, and if it is (SMP <1), this means that the patient does not have viral hepatitis

As shown in Table 2 that out of (50) patients there was only (9) patients.

Table 2 Frequencies of patients who has viral hepatitis or not with its percentage

SMP					
		Frequency	**Percent**	**Valid Percent**	**Cumulative Percent**
Valid	.004	2	4.0	4.0	4.0
	.006	1	2.0	2.0	6.0
	.008	3	6.0	6.0	12.0
	.009	4	8.0	8.0	20.0
	.010	2	4.0	4.0	24.0
	.011	4	8.0	8.0	32.0
	.012	1	2.0	2.0	34.0
	.013	3	6.0	6.0	40.0
	.015	1	2.0	2.0	42.0
	.016	3	6.0	6.0	48.0
	.018	1	2.0	2.0	50.0
	.019	3	6.0	6.0	56.0
	.020	1	2.0	2.0	58.0
	.021	1	2.0	2.0	60.0
	.022	2	4.0	4.0	64.0
	.023	3	6.0	6.0	70.0
	.024	1	2.0	2.0	72.0
	.025	1	2.0	2.0	74.0
	.029	1	2.0	2.0	76.0
	.033	1	2.0	2.0	78.0
	.045	1	2.0	2.0	80.0
	.073	1	2.0	2.0	82.0

1.209	1	2.0	2.0	84.0
1.320	1	2.0	2.0	86.0
1.504	1	2.0	2.0	88.0
1.505	1	2.0	2.0	90.0
1.536	1	2.0	2.0	92.0
1.722	1	2.0	2.0	94.0
1.780	1	2.0	2.0	96.0
1.784	1	2.0	2.0	98.0
2.037	1	2.0	2.0	100.0
Total	**50**	**100.0**	**100.0**	

Figure 1 below shows the shape of normality distribution of data focused on people who do not have viral hepatitis.

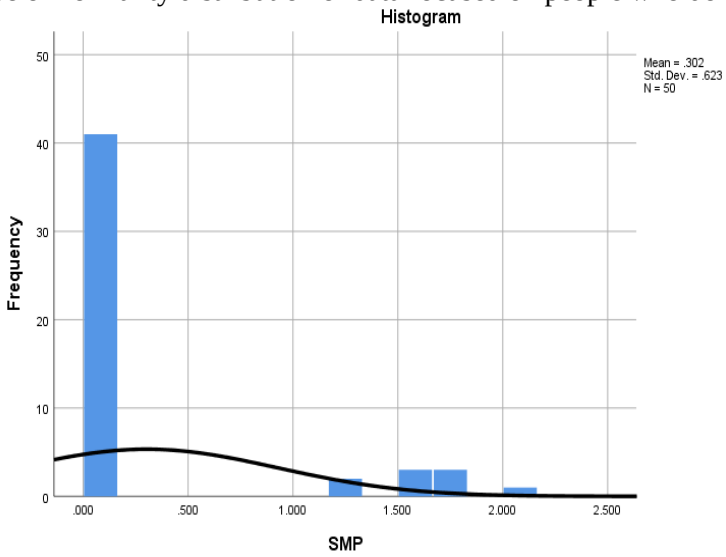

Fig. 1

The ANOVA table shows the averages of patients with viral hepatitis and not infected are not different.

Table 3 ANOVA Table

ANOVA					
SMP					
	Sum of Squares	**df**	**Mean Square**	**F**	**Sig.**
Between Groups	1.755	1	1.755	27.287	.000
Within Groups	.836	13	.064		
Total	**2.591**	**14**			

Table 4 Chi-Square Test

Chi-Square Tests			
	Value	**df**	**Asymptotic Significance (2-sided)**
Pearson Chi-Square	11.324[a]	30	0.020
Likelihood Ratio	10.056	30	0.030
N of Valid Cases	16		

a. 62 cells (100.0%) have expected count less than 5. The minimum expected count is .00

By using Chi – Square test we find that patients data are independent and homogeneous. The data represent (78) cases for the purpose of measuring their infection with viral hepatitis or not. Table 5 indicate that the mean infected is (0.17353), with (0.446451) standard deviation. Also, the data is normally distributed by the values of (Skewness = 1.356 & Kurtosis = 1.736) which should be in the range (± 1.96).

Table 5 Descriptive Statistics for infections in dogs

Statistics		
SMP		
N	Valid	78
	Missing	0
Mean		.17353
Std. Deviation		.446451
Skewness		1.356
Std. Error of Skewness		.272
Kurtosis		1.736
Std. Error of Kurtosis		.538

*If it is (SMP >1), this indicates that the case has viral hepatitis, and if it is (SMP <1), this means that the case does not have viral hepatitis

Table 6 shows the frequencies, percent, valid percent, & cumulative percent of (78) cases the higher case were in (0.032) with six frequencies, then (0.010) with five frequencies, the four frequencies was in two cases (0.014 & 0,031), three frequencies was in four cases (0.010, 0.016, 0.022, & 0.034), finally two frequencies was in (11) cases (0.012, 0.013, 0.017, 0.018, 0.019, 0.023, 0.029, 0.033, 0.042, 0.043, & 0.080), the sixty remaining cases with one frequency.

Table 6 Frequencies of cases who has viral hepatitis or not with its percentage in dogs

SMP					
		Frequency	**Percent**	**Valid Percent**	**Cumulative Percent**
Valid	.010	3	3.8	3.8	3.8
	.011	5	6.4	6.4	10.3
	.012	2	2.6	2.6	12.8
	.013	2	2.6	2.6	15.4
	.014	4	5.1	5.1	20.5
	.016	3	3.8	3.8	24.4
	.017	2	2.6	2.6	26.9
	.018	2	2.6	2.6	29.5
	.019	2	2.6	2.6	32.1
	.020	1	1.3	1.3	33.3
	.021	1	1.3	1.3	34.6
	.022	3	3.8	3.8	38.5
	.023	2	2.6	2.6	41.0
	.024	1	1.3	1.3	42.3
	.025	1	1.3	1.3	43.6
	.027	1	1.3	1.3	44.9
	.029	2	2.6	2.6	47.4
	.031	4	5.1	5.1	52.6
	.032	6	7.7	7.7	60.3
	.033	2	2.6	2.6	62.8
	.034	3	3.8	3.8	66.7
	.038	1	1.3	1.3	67.9
	.042	2	2.6	2.6	70.5
	.043	2	2.6	2.6	73.1
	.052	1	1.3	1.3	74.4
	.054	1	1.3	1.3	75.6
	.055	1	1.3	1.3	76.9
	.059	1	1.3	1.3	78.2
	.062	1	1.3	1.3	79.5
	.063	1	1.3	1.3	80.8
	.066	1	1.3	1.3	82.1
	.070	1	1.3	1.3	83.3
	.073	1	1.3	1.3	84.6
	.080	2	2.6	2.6	87.2
	.234	1	1.3	1.3	88.5
	.346	1	1.3	1.3	89.7
	.430	1	1.3	1.3	91.0
	1.016	1	1.3	1.3	92.3
	1.044	1	1.3	1.3	93.6
	1.097	1	1.3	1.3	94.9
	1.220	1	1.3	1.3	96.2
	2.011	1	1.3	1.3	97.4
	2.014	1	1.3	1.3	98.7
	2.083	1	1.3	1.3	100.0
	Total	**78**	**100.0**	**100.0**	

The ANOVA table shows the averages of patients with viral hepatitis and not infected are not different (Table 7). By using Chi – Square test we find that cases data are independent and homogeneous (Table 8).

Table 7 ANOVA Table for infection in dogs

ANOVA					
SMP					
	Sum of Squares	**df**	**Mean Square**	**F**	**Sig.**
Between Groups	1.945	1	1.945	35.852	.000
Within Groups	.217	4	.05425		
Total	**2.162**	**5**			

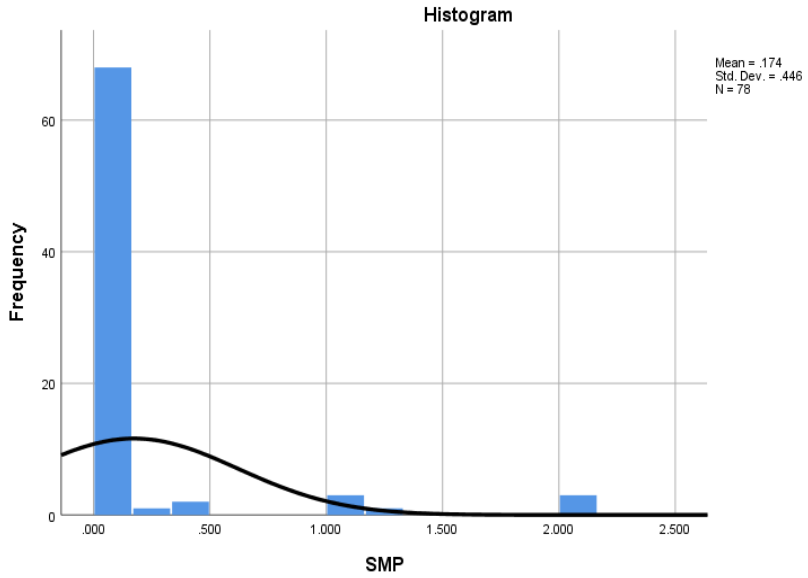

Fig. 2 The shape of normality distribution of data focused on cases who do not have viral hepatitis in dogs

Table 8 Chi – Square Test for infections in dogs

Chi-Square Tests			
	Value	**df**	**Asymptotic Significance (2-sided)**
Pearson Chi-Square	14.514[a]	33	0.020
Likelihood Ratio	13.176	33	0.030
N of Valid Cases	16		

a. 62 cells (100.0%) have expected count less than 5. The minimum expected count is .00

From Table 9 below the sample size of human cases is less than animal cases, this fact affected on other statistical indicators where, the mean and standard deviation of animal cases where less than human, this means that the values of the animal cases are less dispersed than the human case, and this case is calculated in favor of the animal data, also, animal data are normality than human, according to the skewness and kurtosis scales.

Table 9 comparison of Descriptive Statistics

Indicators	**Human**	**Animal**
Sample Size	50	78
Mean	0.30214	1.7353
Standard Deviation	0.622662	0.446451
Skewness	1.827	1.356
Kurtosis	1.594	1.736

Table 10 below shows the cases and its frequencies, which seems that dogs cases has their frequencies much better than human.

Table 10 The cases & its frequencies for human and dogs

Human		**Animal**	
Cases	**Frequencies**	**Cases**	**Frequencies**
-	-	0.032	6
-	-	0.011	5
0.009	4	0.014	4
0.011		0.031	
0.008		0.010	
0.013		0.016	
0.016	3	0.022	3
0.023		0.034	
0.004		0.012	
0.010	2	0.013	
0.022		0.017	
-	-	0.018	
-	-	0.019	
-	-	0.023	
-	-	0.029	
-	-	0.033	2
-	-	0.042	
-	-	0.043	
-	-	0.080	

Table 11 below shows although the values of the two tests were significant, but the value of the animal test titration were higher than human.

Table 11 F – Statistics and Chi – Square test

	Human	Dogs
F – Statistics	27.287	35.852
Chi – Square Test	35.852	14.514

DISCUSSION

Different patterns are indicative of acute or chronic disease in a carrier and are used in conjunction with qualitative assay results to make a serological diagnosis of hepatitis B virus infection. The antigen-antibody system is used to diagnose hepatitis B infection, track the progression of the illness, and oversee treatment**.** Analysis of numerous targets (antibodies, antigens, and DNA) is necessary to fully understand the patterns and stages of HBV infection. In addition, molecular diagnostics alone cannot be used to study the patterns of hepadnavirus infection in cats and dogs (Lee, J.H. et al., 2021).
In the current study indicated that the dogs in Samarra higher infected than human this may be differences in sample size between human and dogs.

A local study in Nineveh Province in 2020 included 78 dogs indicated that the Dogs have a clear case of hepatitis B, which has a serious effect on affected dogs' liver function [5]. While In a 2023 study conducted in Italy, 600 serum samples from domestic dogs were used to test for the canine hepatitis virus. The results showed that the virus was present in household dogs at a prevalence of 10.0% overall, with higher prevalence in younger and older dogs (Fruci, P. et al., 2023).

CONCLUSION

The Samarra Province of Iraq has a significant amount of canine hepatitis B evidence. As a recommendation, it is crucial to conduct additional research on the prevalence of the Hepatitis B virus in other regions of Iraq, including by using molecular techniques.

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