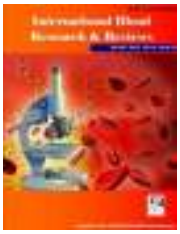

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Haematological Changes Associated With Hepatitis E Virus Seropositivity among Human and Animal Subjects in Plateau State, Nigeria

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Authors' contributions

This work was carried out in collaboration between all authors. Author SAJ designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Authors SAJ and SEA managed the analyses of the study. Authors SAJ and SEA managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Aim: To investigate the haematological manifestations of Hepatitis E Virus (HEV) seropositivity.
Study Design: Cross sectional study. We included 592 subjects in the study; (426 humans and 166 animals). Humans were categorized into 4 groups: apparently healthy (190), pregnant women (108), HIV positive patients (80), and animal handlers (48). The animals were made up of pigs (67), goats (43), sheep (19) and cattle (37).
Methodology: Blood samples were collected and analyzed for HEV antibodies (IgG and IgM) using ELISA technique. Human samples were analyzed using the Abacus Junior Auto Haematology Analyser while the animal Samples were analyzed using Mindray BC-28000Vet Auto Haematology Analyser. Results obtained were analyzed using SPSS version 17.0 statistical software.
Results: Haematological manifestations of HEV seropositivity revealed a significant decrease in Haematocrit (HCT) value among pregnant women (IgG; *P*=.008, 28.9±2.3 vs 36.5±1.7), while among apparently healthy subjects, results revealed a reduced Red Blood Cells (RBC) count (IgG; *P*=.003, 4.2±0.2 vs 4.9±0.2), Platelets (PLT) count (IgG;

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P=.013, 162.4±8.3 vs 205.4±14.6) and HCT (IgM; *P*=.011, 34.1±6.6 vs 40.9±0.4) value, but a raised granulocyte (GRA) count (IgM; *P*<.001, 11.0±6.9 vs 2.9±0.1; IgG; *P*=.006, 3.9±0.5 vs 2.3±0.1) was observed. Among animal subjects; PLT was significantly associated with a raised count (IgM; *P*<.001, 614.5±198.8 vs 328.5±14.7), while GRA recorded a decreased count (IgG; *P*=.023, 4.8±0.8 vs 9.0±0.8). With regards to animal species; raised platelet count (IgM; *P*=.01, 662.5±229.9 vs 349.5±25.4 and IgG; *P*=.001, 797.4±299.9 vs 344.8±23.2), as well as decreased granulocyte count (IgG; *P*=.006, *P*=4.7±0.8 vs 13.6±1.2) was associated with HEV seropositivity among pigs.

Conclusion: Data suggest that HEV Infection results in abnormal changes in some haematological parameters, warranting further attention and research. Haematological approaches should be considered in future studies and diagnosis of HEV infection.

Keywords: Hepatitis E; haematological changes; human; animals; Nigeria.

1. INTRODUCTION

Hepatitis E virus (HEV) was identified as the cause of recurrent large epidemics of hepatitis that occurred regularly during monsoon rains in countries in Southern Asia [1]. Subsequently the virus was sequenced and found to cause large epidemics among refugee populations in Africa and Asia, whose drinking water was faecally contaminated [2,3]. Recently, sporadic cases of hepatitis from HEV infection have been reported among persons living in higher income countries in Western Europe, Japan, China, and North and South America [4]. Hepatitis E was first documented in New Delhi in 1955 when 29,000 cases of icteric hepatitis occurred following the contamination of the city's drinking water [2]. The first experimental evidence for the existence of an additional waterborne hepatitis agent (HEV) was reported in 1983 [5].

Hepatitis E virus (HEV) infection is a significant global public health concern and is often associated with particularly high mortality rates in pregnant women [6]. HEV is transmitted primarily by the faecal-oral route or through contaminated water [7]. It can also be transmitted across species between humans, pigs, boars, deer, chickens, and rabbits [8]. HEV antibodies have been found in pigs, rats, cats, and cattle [9,10], with pigs identified as the most important reservoirs. Evidence has shown that veterinarians working with pigs were at increased risk of acquiring HEV infection [11]. Pigs serve as an important reservoir for HEV, and exposure to pigs may pose a risk of zoonotic infection. Global distribution of HEV infection follows socioeconomic status. The prevalence of antibody to HEV in suspected or documented endemic regions has been much lower than expected (3-26%) [5]. The prevalence of antibody to HEV in non endemic regions (like the US) has been much higher than anticipated (1-3%). HEV infections account for >50% of acute sporadic hepatitis in some high endemic areas. In animal studies carried out in Somalia, Tajikistan and Turkmenistan (endemic regions); 29-62% of HEV prevalence was observed in cattle, 42% to 67% in sheep and goats in Ukraine in non-endemic geographic areas [12].

HEV is a small non enveloped, spherical positive strand RNA virus and the only known agent of enterically transmitted (ET), none A, non B (NANB) hepatitis (ET-NANB hepatitis) [13]. HEV is the only member of the genus Hepavirus and is placed in the family Hepaviridae [14,15].

It is approximately 27-34nm in diameter. The virus has a polyadenylated, single stranded RNA genome, approximately 7.2 kilo bases in length with a positive polar and a cap at its 5^{l}

end. It consists of three open reading frames, ORF-1, ORF-2 and ORF-3 of these, ORF 2 encodes the major capsid protein of the virus and the ORF-3 encodes a small protein of unknown function [16].

Most experimental studies of HEV currently, investigate only virological and hepatic parameters. However, there is limited information available describing the extra-hepatic manifestations of HEV infection, including a few reports highlighting other clinical findings, such as pancreatitis, thrombocytopenia, aplastic anaemia, acute thyroiditis, glomerulonephritis, and neurologic disorders, as reviewed by Dalton et al. [17]. In order to obtain a preliminary understanding of the effect of HEV on haematological profile, of both human and animals, a descriptive study was conducted on the blood of subjects. This article describes, for the first time in Nigeria, haematological disorders that could be interpreted as extra-hepatic manifestations of HEV infection. Haematological approaches should be considered in future studies of HEV infection.

2. MATERIALS AND METHODS

2.1 Study Area

The research was carried out in Plateau State with its capital, Jos. The state is located in the North Central Region of Nigeria. Jos is situated on latitude 9.5ºN and longitude 8.5ºE, and is 4000 feet above sea level. Principally, the state experiences two types of seasons (dry and rainy seasons), with modifications resulting from its high altitude.

Plateau state comprises of seventeen Local Government Areas and three geographical zones. The populations are predominantly farmers and public workers. This project was carried out amongst rural dwellers, students, farmers, public workers, animals (Pig, Cattle, Sheep and Goat) from six (6) Local Government Areas (Jos North, Jos South, Pankshin, Bokkos, Langtang North and Langtang South), representing the three geographical zones.

2.1.1 Study population, study design and sample size

The populations for this study were chosen from the representative zones of Plateau state. Participants were chosen according to a stratified, multistage algorithm to produce a representation of the populace. Extensive efforts were made to ensure high participation rates, i.e. through the hospital authorities, village heads and churches by announcements and encouraging the people to participate.

The study population included: apparently healthy, pregnant women, HIV positive patients, animal handlers and animals (pig, cattle, sheep, and goat).

2.1.1.1 Study design

The study was a Cross sectional study.

2.1.1.2 Sample size

The minimum sample size was calculated from the general formular as described by Thrustfield [18]. The minimum sample size was calculated to be 366 but a total of 592 blood

samples were collected. Sample size greater than the value determined by the formula was used to improve precision estimates of the study.

2.1.2 Inclusion and exclusion criteria

Adults and children of both sexes and animals of all age groups that are sick or apparently healthy in these areas were chosen as the study population. Individuals or animals with drug history of immuno-suppressive therapy or critically ill were excluded from the study.

2.2 Ethical Consideration

The study protocol was reviewed and approved by the Ethical Committee of Plateau State Specialist Hospital Jos and the Ethical Committee of Federal college of Veterinary and Medical Laboratory Technology, Vom–Nigeria. All participants endorsed a written informed consent form.

2.3 Collection of Samples

Altogether, 592 blood samples were collected from apparently healthy individuals, imunocompromised hosts (pregnant women, HIV patients) and animals (pig, cattle, sheep, goat) of both sexes and various age groups.

The upper arm of the individual was tied with tourniquet. The antecubital region was disinfected using cotton wool soaked in 70% alcohol and with a sterile needle and syringe; 10ml of blood was drawn by venepuncture through the antecubital vein. The tourniquet was removed and the punctured vein was blocked with dry cotton wool applying moderate pressure to stop bleeding after removing the needle from the vein. The needle was recapped and then discarded.

Ten millilitre (10ml) of blood was collected from the jugular vein of cattle, sheep and goats, while that of pig was collected from the anterior venecava after properly restraining the animals.

2.3.1 Processing and transportation of samples

Five millilitre (5ml) of the collected blood samples were dispensed into EDTA anticoagulant tubes and mixed properly and labeled appropriately for hematological profile studies. Aseptic precautions were taken in processing and transportation of samples.

2.3.2 Serum sample

The remaining 5mls of blood was transferred gently into a sterile plain container, and allowed to clot and retract. The serum was separated from the clot as early as possible by centrifugation at 2000rpm for 5minutes as to avoid haemolysis of the red blood cells. The serum samples were transferred safely into a sterile cryovial with the disposable Pasteur pipette and labeled accordingly. The labeled serum samples were transported in cold Ice packed jar to the Laboratory and then stored at -80^{o}C until tested.

2.4 Detection of HEV Antibodies

The serum samples were screened for the presence of Hepatitis E virus IgM and IgG antibodies. The test was carried out using Enzyme-linked immumosorbent assay (ELISA) kits for the qualitative detection of IgG and IgM-class antibodies to hepatitis E virus in serum, according to manufacturer's instruction. The ELISA kits were manufactured by Diagnostic Automation, Inc, Calabasas, USA. Details of the ELISA methods used are as described in the Kit manual.

2.5 Measurement of Haematological Profiles

The hematological parameters considered in this study were; White blood cells (WBC), Red blood cells (RBC), Granulocytes (GRA), Platelets (PLT) and Haematocrite (HCT). The process of complete blood count was determined by the use of automated analyzers.

2.5.1 Human samples

Anticoagulated blood samples collected in Potassium Ethylene Diamine Tetra Acetic Acid (K_2 EDTA) were appropriately homogenized and then analyzed using the Abacus Junior Auto Haematology Analyser (DIATRON Messtechnik Ges. m. B. H., A-1141 Wien, Ameisgasse, AUSTRIA).

2.5.2 Animal samples

Anticoagulated blood samples collected in Potassium Ethylene Diamine Tetra Acetic Acid (K_2 EDTA) were appropriately homogenized and then analysed using Mindray BC-28000Vet Auto Haematology Analyser for Veterinary samples (Bio-Medical Electronics Co. Ltd., Shanzhen, China).

2.6 Data Management and Analysis

Data recorded during sampling and laboratory findings were entered and stored in MS-Excel. The data were thoroughly screened for errors and properly coded before being subjected to statistical analysis using the Statistical Package for Social Sciences (SPSS) version 17.0 statistical software (SPSS, Inc., Chicago, IL, USA). Pearson Chi-square test was used to establish association between serological results and Haematological parameters considered in the study. Descriptive statistics were prepared from the study samples, and results were presented as means±SD or percentage. All P values were based on a two sided test of statistical significance. Significance was accepted at the level of $P<0.05$.

3. RESULTS

3.1 Human Subjects

Table 1 shows the relationship of hematological indices of human subjects with seropositivity. Results revealed that for IgM; Granulocytes (GRA) (P=0.000) and haematocrit (HCT) (P=0.011) were significantly associated with HEV seropositivity among apparently healthy subjects. Granulocyte count was increased with a mean of 11.0±6.9 vs 2.9±0.1, while in contrast, a decreased HCT value was recorded with a mean of 34.1±6.6 vs 40.9±0.4.

Table 1. Relationship of HEV seropositivity with haematological indices among human subjects

Subject	Parameter	HEV status					
		IgM			IgG		
		+ve	-ve	P	+ve	-ve	*P*
Pregnant women	WBC	8.2±1.7	5.3±2.2	0.068	5.8±5.1	5.1±0.3	0.139
	RBC	4.9±0.2	45.1±10.7	0.611	37.2±16.8	49.5±13.7	0.568
	PLT	124.0±19.8	118.5±10.3	0.942	116.7±15.4	119.9±13.7	0.877
	GRA	5.1±1.2	67.1±8.7	0.335	68.9±11.6	63.9±12.3	0.773
	HCT	45.5±4.9	33.2±14.7	0.373	28.9±2.3	36.5±1.7	0.008
HIV +ve patients	WBC	2.4±0.1	4.0±0.2	0.104	4.1±0.2	3.9±0.2	0.894
	RBC	4.7±0.6	64.9±11.9	0.423	77.7±18.7	54.1±15.1	0.325
	PLT	172.0±23.9	133.2±12.8	0.613	110.2±19.1	147.4±16.8	0.149
	GRA	106.0±47.0	46.3±10.7	0.393	54.9±17.3	43.5±13.7	0.600
	HCT	39.0±1.4	45.4±4.9	0.838	39.0±3.1	50.0±8.2	0.271
Apparently healthy persons	WBC	6.5±2.1	6.4±0.5	0.988	5.9±0.4	6.9±0.8	0.218
	RBC	4.1±1.4	4.5±1.6	0.601	4.2±0.2	4.9±0.2	0.003
	PLT	217.3±24.2	184.1±8.9	0.586	162.4±8.3	205.4±14.6	0.013
	GRA	3.9±1.5	3.2±0.1	0.000	3.9±0.5	2.6±1.2	0.006
	HCT	40.3±2.0	40.7±6.5	0.011	40.3±0.7	41.0±0.7	0.428
Animal handlers	WBC	4.4±1.6	5.5±1.5	0.159	5.1±0.2	5.9±0.4	0.059
	RBC	4.9±0.3	4.8±0.6	0.647	4.8±0.1	4.9±0.1	0.668
	PLT	223.8±50.6	214.6±13.2	0.838	227.7±15.8	194.7±19.0	0.196
	GRA	1.8±0.5	6.4±4.0	0.731	2.3±0.2	12.2±9.8	0.195
	HCT	45.3±2.2	41.9±3.9	0.101	41.8±0.6	43.0±1.1	0.302

Values are expressed as mean of three replicates±SEM; Statistically significant at (P<0.05) Key: WBC-White Blood Cells ($x10^9$µ/L); RBC-Red Blood Cells($x10^{12}$µ/L); PLT-Platelets($x10^9$µ/L); GRA-Granulocytes($x10^9$µ/L); HCT-Haematocrit(%); +ve- Positive; - ve - Negative

Table 2. Relationship of HEV seropositivity with haematological indices among animal subjects

Parameter	HEV Status					
	IgM			IgG		
	+ve	-ve	P- value	+ve	-ve	*P*- value
WBC	37.6±11.4	58.3±10.3	0.547	21.8±2.9	62.7±11.1	0.125
RBC	7.3±0.7	7.2±0.3	0.974	8.0±0.8	7.1±0.3	0.264
PLT	614.5±198.8	328.5±14.7	0.000	447.5±122.1	335.8±14.3	0.069
GRA	8.9±2.2	8.3±0.7	0.830	4.8±0.8	9.0±0.8	0.023
HCT	38.6±1.7	35.5±0.7	0.225	33.2±1.8	36.3±0.8	0.119

Values are expressed as mean of three replicates±SEM; Statistically significant at (P<0.05) Key: WBC-White Blood Cells ($x10^9$µ/L); RBC-Red Blood Cells ($x10^{12}$µ/L); PLT-Platelets ($x10^9$µ/L); GRA-Granulocytes ($x10^9$µ/L); HCT-Haematocrit (%); +ve - Positive; - ve - Negative

Table 3. Relationship of hematological manifestation of animal species with HEV transmission

Subject	Parameter	HEV Status					
		IgM			IgG		
		+ve	-ve	P	+ve	-ve	*P*
Pigs	WBC	41.4±13.0	91.3±21.0	0.279	16.6±4.1	92.5±19.9	0.141
	RBC	6.7±0.3	7.3±0.7	0.642	7.4±1.2	7.2±0.5	0.863
	PLT	662.5±229.9	349.5±25.4	0.010	797.4±299.9	344.8±23.2	0.001
	GRA	9.6±2.5	13.1±1.2	0.236	4.7±0.8	13.6±1.2	0.006
	HCT	39.8±1.5	38.2±1.1	0.504	35.9±2.8	38.9±1.0	0.289
Goats	WBC	14.5±3.4	26.9±4.6	0.561	25.9±4.2	26.5±6.3	0.951
	RBC	11.1±5.1	7.7±0.6	0.244	8.6±1.2	7.4±0.7	0.368
	PLT	326.5±103.5	248.4±25.1	0.505	227.2±60.4	264.1±21.9	0.483
	GRA	4.5±2.6	5.1±1.1	0.900	5.1±1.3	5.1±1.4	0.981
	HCT	31.3±6.8	29.5±1.3	0.766	29.9±2.2	29.4±1.5	0.863
Cattle	WBC	-	68.3±26.9	-	-	68.3±26.9	-
	RBC	-	7.0±0.4	-	-	7.0±0.4	-
	PLT	-	392.0±30.9	-	-	392.0±30.9	-
	GRA	-	7.7±1.3	-	-	7.7±1.3	-
	HCT	-	35.6±1.7	-	-	35.6±1.7	-
Sheep	WBC	-	11.1±1.4	-	15.7±4.5	10.6±1.4	0.265
	RBC	-	6.7±0.4	-	6.4±1.5	6.7±0.5	0.863
	PLT	-	316.7±31.5	-	414.5±101.5	305.2±33.0	0.300
	GRA	-	2.9±0.3	-	3.8±1.2	2.8±0.3	0.393
	HCT	-	41.0±0.7	-	44.5±4.5	40.6±0.6	0.094

Values are expressed as mean of three replicates±SEM; Statistically significant at (P<0.05) Key: WBC - White Blood Cells ($x10^9$µ/L); RBC-Red Blood Cells($x10^{12}$µ/L); PLT - Platelets ($x10^9$µ/L); GRA -Granulocytes($x10^9$µ/L); HCT - Haematocri (%); +ve - Positive;- ve - Negative

For IgG; reduced Haematocrit value with mean of 28.9±2.3 vs 36.5±1.7 was significantly associated with HEV seropositivity (P=0.008) among pregnant women. Red blood cells (RBC), Platelets (PLT), and Granulocytes (GRA) were significantly associated (P<0.05) with HEV seropositivity among apparently healthy subjects. Results revealed a reduced RBC and PLT count with (P=0.003, 4.2±0.2 vs 4.9±0.2) and (P=0.013, 162.4±8.3 vs 205.4±14.6) respectively, but a raised granulocyte count with P=0.006, 3.9±0.5 vs 2.3±0.1.

3.2 Animal Subjects

Table 2 (above) represents the relationship of hematological indices of Animal subjects with HEV seropositivity. Results revealed that PLT was significantly associated with a raised count value for IgM seropositivity (P=0.000, 614.5±198.8 vs 328.5±14.7), while GRA was significantly associated with decreased count value for IgG seropositivity (P=0.023, 4.8±0.8 vs 9.0±0.8).

Table 3 (above) represents the relationship of hematological indices of Animal Species with HEV seropositivity. Results revealed that increased platelet count was significantly associated with both IgM (P=0.010) (662.5±229.9 vs 349.5±25.4) and IgG (P=0.001) (797.4±299.9 vs 344.8±23.2) Seropositivity, as well as decreased granulocyte count with IgG Seropositivity (P=0.006) (P=4.7±0.8 vs 13.6±1.2) among pigs. No statistically significant relationship was established with regards to goats and sheep. Cattle recorded a 0% HEV Seropositivity and hence could not be considered.

4. DISCUSSION

Data from this study revealed a significant association between HEV seropositivity and effect on some haematological parameters in humans as well as animals. Among human subjects, HEV infection appeared to induce raised granulocyte count, reduced haematocrit, RBC count, and decreased platelet count. In contrast, an increased platelet count and reduced granulocyte count in animals was observed. Data from this study agrees with the observation made by Fourquet et al. [19] that, HEV can induce severe thrombocytopenia in immunocompetent people. In Pakistan, Shah et al. [20] described the causative role of HEV in the development of aplastic anaemia that might not be ignored.

In the present study, data clearly indicated that granulocyte and platelet count were the haematological parameters found to be highly altered to normal cases. The possible reason behind this finding may be that; viral infections, such as infectious hepatitis may cause severe or protracted neutropenia (granulocyte) due to direct damage of the haematopoietic precursor cells [21,22]. Thrombocytopenia and thrombocytosis occur through either immune mediated platelet destruction with or without immune mediated megakaryocyte damage, or alternatively direct toxicity to megakaryocytes resulting from viral infection of these cells [21,22]. A decrease in a number of peripheral blood platelets can be caused by the effect of platelet and platelet-granulocyte aggregations in response to infection [23].

In a case study, Kishore and Sen [24] described the induction of thrombocytopenia and anemia with Parvovirus B19. This was observed in a child with fatal fulminant hepatic failure. The child was coinfected with hepatitis A and E viruses. The study concluded that his anemia and thrombocytopenia might have aggravated or caused fulminant hepatitis. Amarapurkar and Amarapurkar [25] reported that viral hepatitis has been shown to be associated with various extrahepatic manifestations. They described the extrahepatic

manifestations seen in cases of glomerulonephritis (GN), polyarteritis nodosa (PAN), cryoglobulinemia, thrombocytopenia, agranulocytosis, aplastic anemia and pancreatitis, and that these manifestations recover completely with recovery from viral hepatitis.

In an experimental study by de Carvalho et al. [26], where Six cynomolgus monkeys (*Macaca fascicularis*) were inoculated intravenously with swine HEV genotype 3 that was isolated from naturally and experimentally infected pigs, the Cynomolgus monkeys developed subclinical hepatitis that was associated with haematological changes. All of the infected monkeys reportedly showed severe lymphopenia and discrete monocytosis. The study described haematological disorders that are associated with HEV infection, thus providing an additional parameter for future experimental protocols. In addition, the study observed a progressive reduction in the average neutrophil counts of the inoculated animals compared with their baseline levels. Other uncommon extra-hepatic manifestations, such as thrombocytopenia, anaemia [25], and neurological symptoms, have been previously described in HEV-infected patients [27]. de Carvalho et al. [26] observed Monocytosis and lymphohistiocytic hepatitis in their study, while lymphohistiocytic hepatitis has also been described in a rodent model of infection with HEV genotype 4 [28]. These findings are consistent with the abnormal granulocyte count and also raised platelet count observed among animals in the current study particularly in pigs. Other authors have also observed lymphopenia in patients with hepatitis-virus-associated aplastic anaemia (HAA), suggesting that HAA may be influenced by the immune-mediated destruction of bone marrow [29].

This suggests that infection with viral hepatitis indeed has effect on haematological parameters, and further lays credence to the findings of the current study.

5. CONCLUSION

Infection with HEV results in abnormal changes in some haematological parameters. This study therefore suggests that haematological indices may be explored as a tool to assist in the diagnosis HEV infection. The major strength of this study is that to the best of our knowledge, this is the first report of seroprevalence of HEV and its effect on hematological indices of humans and animals in Nigeria, a country with no previously documented data on epidemiology of HEV infection to date. This study was done in animals to establish the zoonotic connection of HEV. Also to prove that HEV induces abnormal haematological changes in animal species as is observed in humans and that animals are an alternate host. Clearly more data is needed to better evaluate the effects of HEV on haematological parameters. Haematological approaches should be considered in future studies of HEV infection. Abnormal Platelet count appears to be the best biomarker that can work for endemic regions.

CONSENT

All authors declare that the study was approved by the ethics committee of our institution and informed consent was obtained from all participants.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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