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Seroprevalence of Hepatitis E Virus among Domestic Animals 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 KJ managed the literature searches. All authors read and approved the final manuscript.

Original Research Article

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ABSTRACT

Aim: This study was undertaken to determine the epidemiology, seroprevalence and associated risk factors, of Hepatitis E virus (HEV) among domestic animals.
Study Design: Cross sectional epidemiological survey.
Place and Duration: The study was carried out in three geographical zones of Plateau State, over a six month period from July to December, 2012.
Methodology: A total of 166 animal subjects were recruited into the study.
The animals studied were made up of pigs (67), goats (43), sheep (19) and cattle (37). Information was obtained from the animal subject handlers using interviewer administered questionnaire. Blood samples were collected and analyzed for HEV antibodies (IgG and IgM) using Enzyme-linked immunosorbent assay (ELISA) technique. Data obtained were analyzed using SPSS version 17.0 statistical software.
Results: Results revealed an overall prevalence of 24.1% (p<.001) with IgG and IgM accounting for 16.3% and 7.8% respectively. Goats recorded the highest prevalence with 37.2%, followed by Pigs with 32.8% and Sheep with 10.5%, but it is note-worthy that Cattle recorded 0% overall seropositivity. Statistical significant association was observed

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with regard to age (p=.04); animals <1 year old accounted for the highest seroprevalence (21.3%) and least among animals ≥2years old (7.7% OR 0.3; 95%CI0.1-1.1). Seropositivity tends to decreases with increase in age. A similar trend was observed with regard to IgM seropositivity. The significant associated risk factor was; frequency of waste disposal (p<.001) (IgM, OR 39.1; 95% CI 4.9-310.4; IgG, OR 19.9; 95% CI3.9-100.7). Animals that had been vaccinated against other diseases tend to exhibit the least seropositivity compared to animal subjects with no history of any form of vaccination.

Conclusion: Data suggest that HEV remains an under-recognized and significant public health issue in the study area, and prevalent among domestic animals, warranting further attention and research. Preventive public health measures should be reinforced among all communities' particularly domestic animals and a periodic monitoring system set up for control.

Keywords: Hepatitis E virus; animals; seroprevalence; risk factors; Nigeria.

1. INTRODUCTION

Although it is still yet to be fully clarified, pigs are believed to be the natural host for the virus [1-3]. Wild and domestic animals are being identified as potential HEV reservoir [4-6]. Domestic animals have been reported as a reservoir for hepatitis E virus, with some surveys showing infection rates exceeding 95% among domestic pigs [7].

Growing evidence suggest that individuals who work with swine such as pig farmers, Veterinarians and slaughter house workers are at increased risk of acquiring HEV infection [8,9].

HEV infection is a zoonosis mainly seen in humans and pigs [1,8,10,11]. Hepatitis E is prevalent in most developing countries, and common in any country with a hot climate. It is wide spread in Southeast Asia, Northern and Central Africa, India, and Central America. It is spread mainly through fecal contamination of water supplies or food. Outbreaks of epidemic hepatitis E most commonly occur after heavy rain falls and monsoons because of their disruption of water supplies [7].

As for new viral pathogens with animal origins, *hepatitis E virus* (HEV) is responsible for many sporadic waterborne cases and epidemics around the world, as confirmed by the case of the Cruise Ship "Aurora", which took place in 2008 [12]. HEV infection may be asymptomatic in industrialized countries, where it can be considered quite rare, with a tendency toward an increase, possibly mediated by migration flows from endemic countries [13]. Consumption of raw meat of infected animals, in particular pigs, as well as occupations involving contact with pigs or biologic pig materials have been identified as possible routes of transmission.

Prevention is based on knowledge, but very often the processes by which zoonoses emerge and re-emerge are complex and poorly understood [14], mainly because a single event, or a chain of events, that promote the emergence of a disease and/or its evolution into an endemic disease, often vary on a case by case basis, and are affected by several factors such as genetic evolution, environmental conditions, climate changes affecting the vector's distribution, demographic changes, movement of animals, etc. [15].

Accumulating evidence indicated that hepatitis E is a zoonotic disease, and pigs (and more likely other animal species) are reservoirs for HEV. Since animals share the same habitat with humans in the study area, and possibly drink from common source, this may lead to cross contamination. Furthermore; inadequate and poor availability of potable water, especially in rural areas may serve as potential source of transmission.

It is possible that the disease has been thriving unnoticed, most likely because an epidemic has not been documented in Nigeria. There is therefore an urgent need for a research of this nature to provide necessary information for pro-active strategy formulation.

2. MATERIALS AND METHODS

2.1 Study Area

The research was carried out in Plateau State with its capital as Jos, and located in the North Central Region of Nigeria. Jos is situated on latitude 9.5°N and longitude 8.5°E, and is 4000 feet above sea level. Principally, the state experiences two types of seasons (dry and rainy seasons), with modifications resulting from its higher altitude. The annual temperature ranges from 50 to 95°F, while the annual rainfall also ranges from 40 to 70 inches. Plateau state comprises of seventeen Local Government Areas and three geographical zones. The populations are predominantly farmers and public workers.

2.1.1 Study subjects

The animal study subjects comprise; pigs (67), goats (43), sheep (19) and cattle (37).

2.2 Ethical Consideration

The study protocol was reviewed and approved by the Ethical Committee of Federal College of Veterinary and Medical Laboratory Technology, Vom–Nigeria.

2.3 Study Design

Cross sectional epidemiological survey.

2.4 Place and Duration

The study was carried out in three geographical zones of Plateau State, over a six month period from July to December, 2012.

2.5 Data Collection

A well structured Questionnaire based on direct and indirect questions to obtain demographic characteristics such as age, sex, as well as possible associated risk factors was administered and filled by consenting animal owners/handlers before sample collection. Those who could neither read nor write were assisted using the local lingua franca; mainly Hausa and indigenous dialects.

2.6 Sample Size

The minimum sample size was calculated from the general formular as described by Fisher et al. [16] and Thrustfield [17]. Sample size greater than determined by the formula was used to improve precision estimates of the study.

2.6.1 Sampling frame

The sampling frame for the studied animals were; pigs (226), goats (145), cattle (124) and sheep (64) giving a total of 560 animals. The animals were proportionately drawn from each species. The sample size for the study was 166, selected using systematic technique. The author expected a proportion of zero (0%) positive samples with 95% confidence interval. Percentage of positive cases was calculated for each kind of animal.

2.6.2 Sampling technique

A systematic technique by Purposive selection was used to select the study subjects as described by Thrustfield [17].

2.6.3 Sample collection

A total of 166 blood samples were taken and sera were separated aseptically and kept frozen at -20℃ before being sent to our laboratory for testing.

2.7 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 human serum (Manufactured by Diagnostic Automation, Inc, Calabasas, USA) in accordance with the manufacturer's instructions. The results were scored as positive or negative according to the standard procedures recommended by the manufacturer. Positive and negative controls were included in all the ELISA microplates assayed.

2.8 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 different risk factors considered in the study. Descriptive statistics were prepared from the study samples, and results were presented as means±SD or percentage. In order to determine the correlation between the data obtained from the questionnaire and the laboratory results, odds ratios (ORs) and their corresponding 95% confidence intervals (CIs) were calculated using binary logistic regression analysis. This was to determine whether a variable was associated with HEV infection. The Pearson Chi square (χ^2) test was used to compare categorical data, and to evaluate the difference in prevalence between groups in the univariate analysis as well as the statistical significance between relevant variables. All P values were based on a two sided test of statistical significance. Significance was accepted at the level of $P<.05$.

3. RESULTS

3.1 Seroprevalence of HEV

(Table 1) represents the seroprevalence of HEV in animal subjects studied. Results revealed an overall prevalence of 24.1% (p=.001). HEV IgG prevalence was 16.3% while that of HEV IgM was 7.8%. Goats recorded the highest prevalence with 37.2%, followed by pigs with 32.8% and sheep with 10.5%, but it is note-worthy that cattle recorded 0% overall prevalence.

3.2 Analysis of Associated Risk Factors

(Table 2) shows the risk factors associated with HEV prevalence among animal study subjects. Statistical significant association was observed with regard to age (p=.04) with IgG; seropositivity tend to decrease with increase in age. Animals <1 year old accounted for the highest seroprevalence (21.3%) and least among animals ≥2years old (7.7% OR0.3; 95%CI0.1-1.1). A similar trend was observed with regard to IgM seroprevalence. This implies that younger animals had higher risk of infection than older animals.

An assessment of Location with regard to animal seroprevalence, indicated no association between location and seroprevalence (p>.05) and the result was generally equivocal with both IgM and IgG. However, the highest prevalence was recorded in central Plateau with 10.3% (OR2.2; 95% CI0.5-10.3) and least in northern Plateau with 5.0% for IgM. Meanwhile for IgG, the highest prevalence was recorded in northern Plateau at 20.0% and the least in southern Plateau at 13.4% (OR0.6; 95% CI0.2-1.6). There was no statistically significant difference recorded with regard to the effect of vaccination against other diseases on HEV seroprevalence (p>.05). However, animals that had been vaccinated against other diseases tend to exhibit the least seropositivity compared to animal subjects with no history of any form of vaccination which tend to exhibit the highest seropositivity. The results for vaccinated vs not vaccinated animals for both IgM and IgG showed a similar trend with 5.0% vs 8.2% (OR1.7; 95% CI0.2–13.8) for IgM and 15.0% vs 16.4(OR1.1; 95% CI0.3–4.1) for IgG (Table 2).

Results showed that there was no association between previous other diseases suffered and seroprevalence of HEV among the animal subjects p>0.05.

Animal waste disposal method as a risk factor was observed to be strongly associated with HEV seroprevalence in animals studied (p<.001). A similar prevalence trend was observed for both IgM and IgG. Animals whose waste was not removed from their Pen recorded the highest prevalence. The lowest prevalence was recorded by the animals whose wastes were cleaned daily. Considering the prevalence of IgM, animals whose wastes were not cleaned at all were almost forty times more likely to be seropositive than those whose waste were cleaned off daily (OR 39.1; 95% CI4.9-310.4). In the case of IgG, animals whose wastes were not cleaned at all, were approximately twenty times more likely to be seropositive than those whose wastes were cleaned off daily (OR19.9; 95% CI3.9-100.7).

Table 1. Seroprevalence of HEV in relation to animal subjects studied

Antibody	Variables						P- value
	Pigs n=67	Goats n=43	Cattle n=37	Sheep n=19	Total N=166	Prevalence (%) (95%CI)	
IgM	11	2	0	0	13	7.8(4.2-12.0)	0.001
IgG	11	14	0	2	27	16.3(10.8-22.3)	0.001
Total	22	16	0	2	40		
Prevalence (95% C. I.)	32.8 %(22.4-44.8)	37.2%(23.3-53.4)	0% (-)	10.5%(0.0-26.3)	24.1%(18.1-30.7)	24.1	

Table 2. Factors associated with HEV Prevalence in animals studied

Variable	HEV Status							
	IgM				IgG	%	OR(95%CI)	P-value
	No. Pos	%	OR(95%CI)	P-value	No. Pos			
Age in years								
<1	10/94	10.6	1.0	0.104	20/94	21.3	1.0	0.042
1<2	2/33	6.1	0.5(0.1-2.6)		4/33	12.1	0.5(0.2-1.6)	
≥2	1/39	2.6	0.2(0.0-1.8)		3/39	7.7	0.3(0.1-1.1)	
Location								
North. Plateau	3/60	5.0	1.0	0.419	12/60	20.0	1.0	0.597
Central Plateau	4/39	10.3	2.2(0.5-10.3)		6/39	15.4	0.7(0.2-2.1)	
South. Plateau	6/67	9.0	1.9(0.4-7.8)		9/67	13.4	0.6(0.2-1.6)	
Vaccination								
Yes	1/20	5.0	1.0	0.953	3/20	15.0	0.1	1.000
No	12/146	8.2	1.7(0.2-13.8)		24/146	16.4	1.1(0.3-4.1)	
Previous other disease suffered								
Yes	2/37	5.4	1.0	0.783	7/37	18.9	1.0	0.620
No	11/129	8.5	1.6(0.3-7.7)		20/129	15.5	0.8(0.3-2.0)	
Waste disposal								
Daily	1/57	1.8	1.0	0.000	4/57	7.0	1.0	0.000
Weekly	2/86	2.3	1.1(0.2-6.7)		10/86	11.6	1.7(0.5-5.9)	
Bi-weekly	1/6	16.7	2.2(0.1-35.5)		3/6	50.0	13.3(2.0-88.1)	
Monthly	2/7	28.6	8.0(0.7-88.1)		4/7	57.1	17.7(2.9-107.9)	
Not at all	7/10	70.0	39.1(4.9-310.4)		6/10	60.0	19.9(3.9-100.7)	

Key: OR=Odds Ratio, No. Pos=Number positive, CI=Confidence Interval

4. DISCUSSION

Several studies carried out indicate that animals such as pigs, cattle, goat, and sheep are reservoirs of HEV antibodies [9,18]. From this study, there was significant difference (p=.001) between the animal species analysed. This indicates that goats appeared more susceptible to HEV infection compared to the other animal subjects in the study area. This is in contrast with findings of Wang et al. [18] who had a mean positive rate of 78.8% for pigs, 6.3% for cattle and none for goats. The absence of HEV antibodies among cattle in the current study is consistent and in line with the report in Shaxi (China) where HEV was not found in any of the 55 cattle tested [18]. However, this is in contrast to studies carried out in Somalia, Tajikistan and Turkmenistan (endemic regions) where 29-62% of HEV was observed in cattle, 42% to 67% in sheep and goats in Ukraine in non-endemic geographic areas [19]. Of the 70 cows tested in Brazil only 1(1.4%) had HEV, but IgG was not found in any of the 12 sheep and 5 goats tested [20]. Also in contrast, specific antibodies were not detected in goats from Shaxi China [18]. A logical explanation could be that the breed of cattle is less susceptible to HEV infection or that they are resistant to the prevailing HEV strains in the area.

The rate recorded for pigs (32.8%), is comparable to those obtained from studies carried out in Thailand (30.7%) [21], United States of America (34.5%) [22] and Taiwan (37.1%) [8]. The similarity in socioeconomic status and consequently poor sanitary condition, poor methods of keeping pigs and rural settings between Africa and Asia may account for the similarity of results from these two regions. However, when compared to other studies carried out in Brazil (63.6%) [20], Indonesia (72%) [23], China (78.8%) [18], Great Britain (85.5%) [24], the current study rate appeared much lower. This may not be a surprise as these industrialized countries engage more in pig farming and high production of pork in contrast to most part of northern Nigeria where pork consumption is forbidden on religious grounds. However, a much lower rate was reported in Mexico (6.0%) [25], Thailand (13.0%) [25], Canada (18.1%) [21], Argentina (22.7%) [26]. These discrepancies in prevalence rates are likely to be related to the rural-urban differences in study areas. Other reasons may lie in the differences in socioeconomic, cultural, hygienic and climatic factors across geographical divides.

4.1 Animal Waste Disposal

Animal waste disposal as a risk factor was predictably strongly associated (p<.001, both IgM and IgG) with HEV transmission. Non-frequent disposal of animal waste was the most significant risk factor in HEV transmission among confined animals, while animals whose wastes were cleared off daily, appeared least susceptible. This underscores the importance of hygiene and sanitary factor on the farm and other forms of animal housing. This is consistent with findings in similar studies in Indonesia [27] and Ghana [28]. Deplorable sanitation in farms and animal pen may be the logical and most likely reason for the strong association with HEV seroprevalence. This underscores a direct relationship of animal Pen hygiene and HEV seroprevalence as observed from this study.

4.2 Age Factor in Animals

Among the animals studied, HEV seroprevalence appeared to decrease with increase in age, suggesting that younger animals seem to be at higher risk than older ones. The results revealed that animals <1year old are at highest risk. This agrees with the work done by

Wang et al. [18] who reported that piglets had a positivity rate of 20% over adult pigs, thus suggesting that infection may occur early in life.

The detection of HEV RNA in sera shows a very strong influence of the age of animals as shown in a Japanese study [29]. The highest number of viremic animals was observed at 3 months of age. The finding of the current study, also suggests that HEV infectivity in animals decreases with age. This age factor observed among animals in the present study is consistent with the findings of other studies in Spain [30], Netherlands [31], Canada [32], and UK [33]. The logical reasoning behind this may be that younger animals are more susceptible to infection and perhaps susceptibility reduces with age. However in another study in Japan, Takahashi et al. [34] reported a contrary finding that positivity increases with age. The reason for this divergence is not properly understood.

Other animal risk factors like; location, other vaccinations given and previous other disease(s) suffered could not be linked with HEV seropositivity as they were not found to be statistically significant. However, animals that had been vaccinated against other animal diseases appeared to be less susceptible to HEV infection.

The likely logical explanation for this less susceptibility could be that vaccinations with other antigens or previous infection with other strains of HEV likely conferred cross protection against subsequent infections from different strains of the same virus [35].

5. CONCLUSION

This study shows that HEV is higher in animals of ages less than 1 year old and least among animals ≥2. Goats had the highest seroprevalence followed by pigs and sheep, with no HEV prevalence in cattle. None frequent disposal of animal waste was strongly associated with HEV transmission among confined animals. This underscores the importance of hygiene and sanitary factor on the farm and other forms of animal housing Location, vaccination against other diseases, history of previous and diseases outbreaks among the animal species appeared to be un associated with seropositivity among the animals studied. Therefore Goats and Pigs rather than, Sheep or Cattle, may act as a natural reservoir of HEV infection in the study area.

This study hypothesize that both zoonotic and anthroponotic transmission of a virulent HEV is occurring extensively in rural villages. Findings of this study do add to the growing evidence that hepatitis E may be a zoonosis and specifically to the concept of it as an occupational infection of livestock workers. HEV remains an under-recognized and significant public health problem in Plateau state and warranting further attention.

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COMPETING INTEREST

Authors have declared that no competing interest exists.

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