Original Research Article

SERO-PREVALENCE OF HEPATITIS E VIRUS IN SOME DOMESTICATED ANIMALS IN EKITI STATE, NIGERIA

ABSTRACT

Aim: This study is aimed to determine the prevalence of Hepatitis E virus (HEV) total antibody in some domesticated animals in Ekiti State, Nigeria.

Study Design: The study was a cross-sectional study conducted at Ekiti State, Nigeria.

Place and Duration of the Study: The study was conducted at Ekiti State, Nigeria between October, 2023 and January, 2024. Sample testing and analysis of serological results were performed at Department of Microbiology, Federal University of Technology Akure, Ondo State, Nigeria.

Methodology: One hundred and eight-six (186) serum samples from four (4) animal species were randomly collected. The serum samples were obtained from the following animals in the study area using the technique of venipuncture: Dogs (n=56), Pigs (n=29), Sheep (n=59) and Goat (n=42). Blood samples were collected and analyzed for HEV total antibody using Human (HEV-Ab) enzyme-linked immunosorbent assay (ELISA).

Results: The overall seroprevalence of HEV total antibodies was 41.94%. Among the four animal species sampled, Pigs had the highest seroprevalence (72.4%). Out of the five Local Government areas, Ido-Osi had the highest HEV seroprevalence (53.57%). The age group ranging from >12-≤18 months had higher HEV prevalence (29.41%) among the age group. All age groups of the domesticated animals had statistically significant differences (P = .05). Goats had a considerably higher seroprevalence of HEV total antibody (73.33%) than other sampled pregnant domesticated animals in the study. The HEV prevalence among the sampled pregnant domesticated animals was statistically significant (P = .05). The prevalence of HEV were 86.41% and 69.23% higher in all domesticated animals sampled in the study areas where the risk factors (unclean environment, dirty water, mixed and extensive management practices) were more prevalent.

Conclusion: Data suggest that HEV remains an under-recognized and significant public health problem, warranting further attention and research.

Keywords: Hepatitis E virus, seroprevalence, ELISA, risk factors

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1. INTRODUCTION

The Hepatitis E virus (HEV) is a significant contributor to acute hepatitis in humans on a global scale. Globally, 20 million individuals are infected annually, with 3.3 million illnesses and 44,000 deaths (1). HEV infection remains common in Nigeria, including recent outbreaks (2). HEV is classified as a member of the Hepeviridae family and is characterized by its non-segmented, non-enveloped structure and single-stranded ribonucleic acid (RNA) composition (3;4). Public health research links HEV detection and contamination in food animals (meat) to zoonotic disease and food safety (5;6). Companion animals and livestock are typically considered HEV zoonotic disease reservoirs due to public health concerns (7).
HEV infection is self-limiting and asymptomatic, causing diarrhea, epigastric pain, nausea, vomiting, hepatomegaly, and splenomegaly. Diagnosing HEV necessitates the use of both molecular and serological approaches to confirm infection and assess the treatment response in individuals with chronic illnesses. Multiple serological and molecular methods exist for detecting HEV, which can be employed to identify viral proteins and/or genomes. Tests for HEV-specific antibodies in the patient's serum are available commercially today in various test formats. Common methods are: Western blot, line immunoassay (LIA), enzyme-linked immunosorbent assay (ELISA), or enzyme immunoassay (EIA). HEV infection management is based on its clinical presentation. The clinical presenting spectrum includes asymptomatic infections, icteric and anicteric acute hepatitis, as well as chronic hepatitis or liver failure. HEV infection typically resolves on its own without the need for antiviral treatment. Treating icteric or anicteric viral hepatitis mostly involves conservative care, emphasizing the natural resolution of the disease. Ribavirin has become increasingly recognized for its effectiveness in treating HEV infection in recent years. Ribavirin is a prodrug that functions as a guanosine analogue. Consequently, the primary objective of this study is to determine the seroprevalence of HEV antibodies among some domesticated animals within the geographical region of Ekiti State, Nigeria.

2. MATERIAL AND METHODS

2.1 Sample location and Study Population

The study involved random selection of some domesticated animals in a selected study Area in Ekiti State, Nigeria. Adult and small animals (Dogs, Pigs, Goats and Sheep) of both sexes and all age groups that are pregnant, sick or apparently healthy in these areas were randomly selected as the study population.

2.2 Demography Data Collection

A questionnaire was employed to collect demographic data prior to sample collection, including factors such as age, sex, and pregnant animals, as well as other relevant information. A distinct participant animal identification number (PIDN) was assigned to each questionnaire. The pre-test questionnaires were directly administered to the individuals responsible for owning and handling the animals. The completeness of all completed questionnaires was assessed.

2.3 Sampling and Sample Collection

One hundred and eight-six (186) serum samples from four animal species were randomly collected. The serum samples were obtained from the following animals in the study area using the technique of venipuncture; Dogs (n=56), Pigs (n=29), Sheep (n=58) and goat (n=42). The animals included in this present study were domestically bred and kept in rural households in Ekiti State, Southwestern Nigeria. A blood sample of up to 5 mL was obtained from each animal species and the blood was drawn from the cephalic and jugular vein using 5ml needle and syringe, after properly restraining of the animals. The blood samples were

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kept in a Serum-separating tubes (without anticoagulant reagent) at room temperature (20^0C) until visible clot retraction. After being centrifuged at 3000× g for 10 minutes at 2-8^{0}C within 30 minutes of collection, the sera were separated and stored at −80^{0}C until analysis.

2.4 Serology

All 186 Animal sera were tested following manufacturer instructions with the Human (HEV-Ab) ELISA Kit (ELISA KITidea Medical Technology Company, Shanghai, China) that uses a recombinant antigen highly conserved between different HEV strains.

2.5 Statistical Analysis

Data were analyzed using Statistical Package for Social Sciences (SPSS) to compare HEV positive and negative samples. Prevalence was illustrated as a percentage. Using Pearson chi-square method was used to show the relationship between the risk factors and The threshold for statistical significance was set at p=.05 (95% confidence intervals). Microsoft Excel 2013 was used to draw the bar charts.

3. RESULTS AND DISCUSSION

3.1 RESULTS

Figure 1 shows the Overall Sero-prevalence of HEV in some domesticated animals in the selected study areas in Ekiti State Out of one hundred and eight-six serum sampled collected from five species of animals, 78 (41.9%) were positive and 108 (58.0%) were negative for Hepatitis E Virus.

Figure 2 shows the Seroprevalence of HEV among the Sampled Domesticated Animals in the Selected Study Areas in Ekiti State, Nigeria. Among the five animal species that were sampled, it was observed that Pigs had the highest percentage of seroprevalence (72.4%). This was followed by Goat (55%) and Dog (32.14%). On the other hand, sheep had the lowest prevalence (30.51%). The Chi-square test statistic (X^2) is 18 with a p-value of 0.021. The p-value is much lower than .05, indicating the seroprevalence of total antibody positivity varied significantly among the domesticated animals sampled in the designated study area.

Figure 3 shows the Sero-prevalence of HEV in the selected Local Government Areas in Ekiti State, Nigeria. Out of the five Local Government Area, Ido- Osi has the highest percentage Seroprevalence of HEV (53.57 %), followed by Ado with the percentage Seroprevalence (46.94 %) and Ilejeme has the least percentage seroprevalence (26%) among the five Local Government. Statistically significant differences (p=.04) were observed among the selected Local Government Area.

Figure 4 shows the Seroprevalence of HEV total antibody in the domesticated Animals based on age distribution across the selected study area in Ekiti State, Nigeria. The age group >24 had the highest seroprevalence of total Antibody, with a prevalence (100%). This was followed by the animals in the age groups >18-≤24 and >6-≤12, which had a prevalence (47.83%) and (47.13%) respectively. The age group between >12-≤18 had a prevalence rate (29.41%), while the age group of 0-<6 had the lowest prevalence (25.71). The Chi-square test statistic (X^2) is 36 with a p-value of 0.015. The p-value is than .05, indicating all age groups of the domesticated animals exhibited statistically significant differences

Figure 5 shows the Distribution of HEV total antibody based on sex of domesticated Animals in the selected study area in Ekiti State, Nigeria. The prevalence of total antibodies was higher in female (62.82%) than male (37.18%) domesticated animals. The HEV total antibody prevalence was significantly different (p= .01) based on sex.

Figure 6 shows the distribution of HEV total antibody among the pregnant domesticated Animals in the selected study area in Ekiti State, Nigeria. Goats had significantly high

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prevalence of total antibody (73.33%), followed by dogs (55.56%), while sheep (44.44%) had the least prevalence. The difference in overall antibody prevalence among pregnant domesticated animals was statistically significant (p=.01).
Figure 7 shows the HEV associated risk factors in the Study Areas in Ekiti, Nigeria. The frequency of HEV was found to be high in all domesticated animals sampled in the study areas where the risk factors (unclean environment, dirty water, mixed and extensive management practices) were more prevalent.

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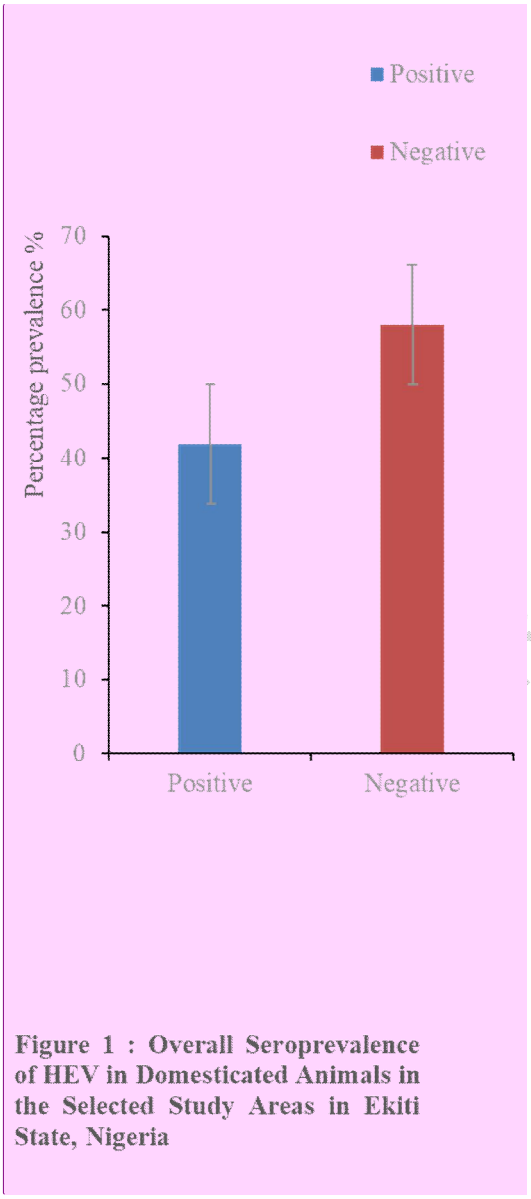
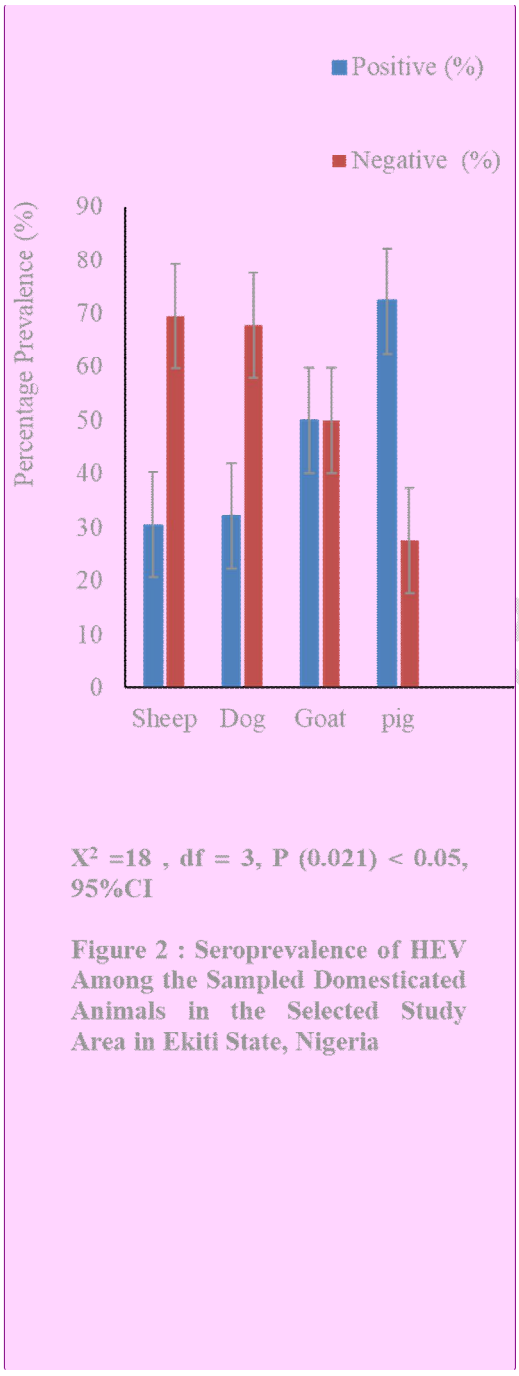
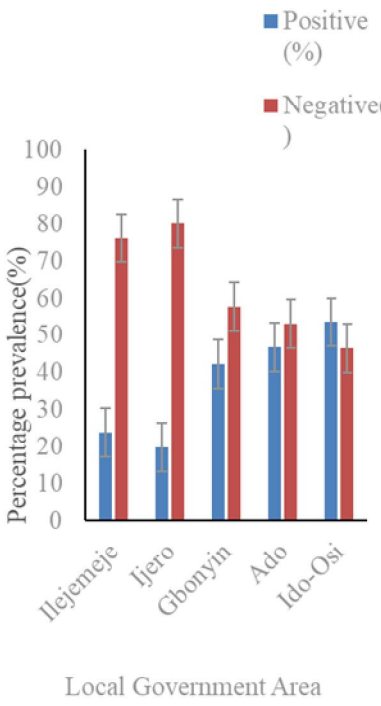


Figure 1 : Overall Seroprevalence of HEV in Domesticated Animals in the Selected Study Areas in Ekiti State, Nigeria



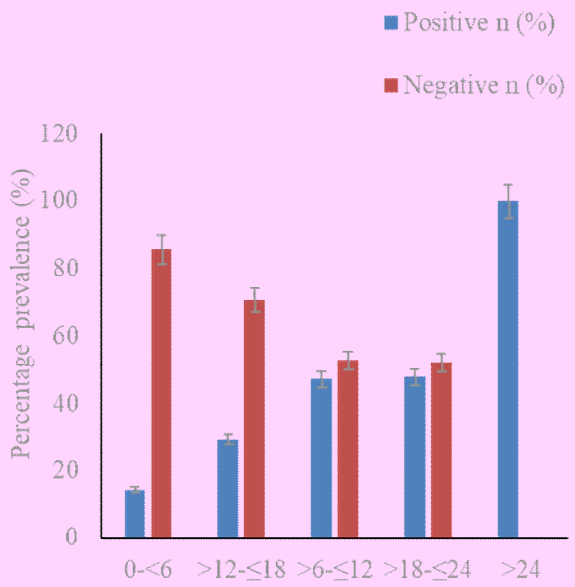
X^2 =18 , df = 3, P (0.021) < 0.05, 95%CI

Figure 2 : Seroprevalence of HEV Among the Sampled Domesticated Animals in the Selected Study Area in Ekiti State, Nigeria



X^2 -45 , P-value (0.041) < 0.05, df= 4, 95%CI

Figure 3: Seroprevalence of HEV in the Selected Local Government Areas in Ekiti State, Nigeria



X^2 = 36 , P-value (0.015) < 0.05, df= 4, 95CI

Figure 4: Seroprevalence of HEV total antibody in the domesticated Animals based on age distribution across the selected study areas in Ekiti State, Nigeria

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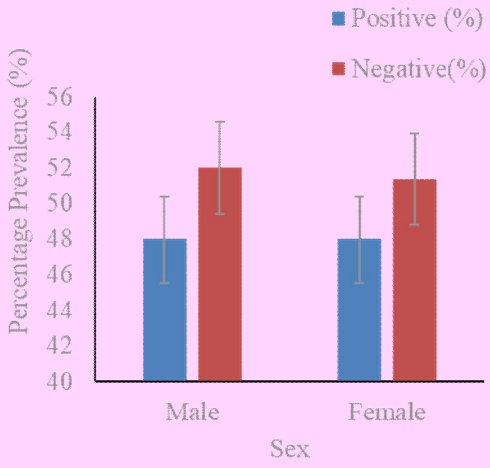
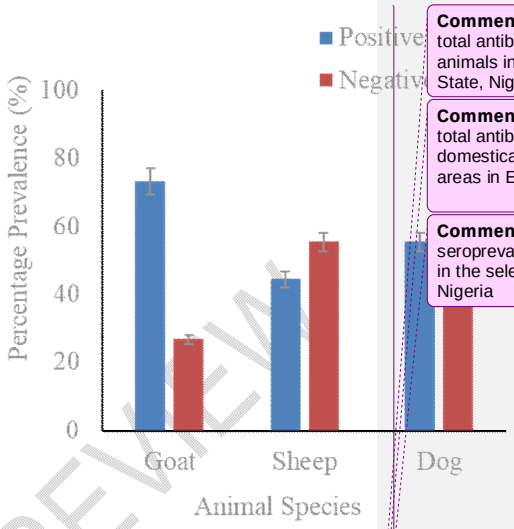


X^2=18 , P-value (0.01) < 0.05, df= 1, 95%CI

Figure 5: Distribution of HE V Total Antibody based to the sex of Domesticated Animals in the selected study area in Ekiti State, Nigeria



X^2 = 27, P-value (0.01) < 0.05, df= 2, 95%CI

Figure 6: Distribution of HE V Total Antibody among the pregnant Domesticated Animals in the selected study area in Ekiti State, Nigeria

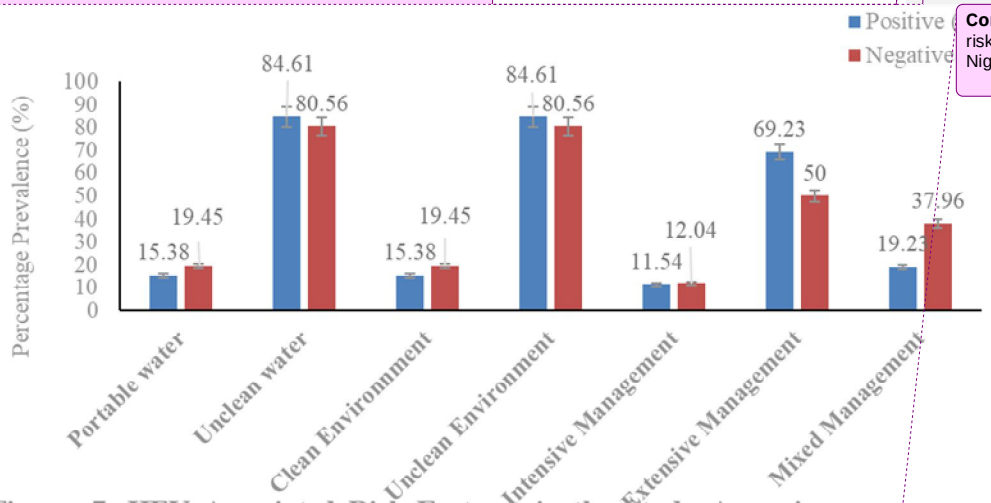


Figure. 7: HEV Associated Risk Factors in the Study Areas in Ekiti State, Nigeria

Comment [B84]: Figure 5: Distribution of HEV total antibody based to the sex of domesticated animals in the selected study areas in Ekiti State, Nigeria

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Comment [B87]: Figure 7: HEV associated risk factors in the study areas in Ekiti State, Nigeria

3.2 DISCUSSION

Globally, there is significantly less research on hepatitis E virus (HEV) infection compared to other types of hepatitis viral infection. The present study investigated the seropositivity of HEV in domesticated animals (dogs, pigs, goats and sheep). The study revealed that the seroprevalence of HEV in Ekiti State is 41.9%, which is in contrast to the seroprevalence reported by (8) in Plateau State, Nigeria where it was 24.1% ($p<0.001$) among domestic animals. This is higher than the 12.2% overall prevalence of anti-HEV reported by (9) in Ibadan, Nigeria and 15.0% and 3.8% anti-HEV total antibodies, reported by (10) in Osun State, Nigeria.

The present study showed that pigs had the highest seroprevalence among the sampled animals, which was slightly higher than that reported by (11) in Plateau State, Nigeria, where HEV prevalence was higher in pigs (65.7%) than humans (10.8%). A possible reason for this observed disparity could be linked to the high exposure of pigs in the study areas to contaminated feed, water, and other risk factors.

In the present study, sheep seroprevalence was slightly higher than in Spain (2.1%) (12) and (2.6%) (13), in contrast to the high seropositivity reported in Portugal (16.6%) (7) and Italy (21.3%) (14) and (21.6%) (15). These findings are slightly lower than the seroprevalence observed in the present study. These results confirm that small ruminants are naturally exposed to HEV in Ekiti State, Nigeria. Comparing cross-study data should be made with caution due to differences in epidemiological background, study designs, serological methods, and animal ages. Recent study results for sheep are slightly higher than those of (15) from Italy, who reported (21.6%) HEV positive results in sheep serum samples and (16) from USA, who presented 16% HEV-positive results in serum samples of mature goats. High levels of HEV seropositivity were seen in sheep and goats due to these animals undergo breeding practices in rural regions of the nation. Typically, sheep and goats choose to graze on unenclosed pastures and meadows situated outside the settlements. Frequently, these animals drink water from natural springs and untested sources. These factors lead to an increased risk of HEV infection. On the other hand, the high HEV seropositivity among goats and sheep are similar to that found in Bulgarian wild boars (40.8%) (17) and East Balkan swine the only aboriginal pig breed in Bulgaria (82.5%) (18). The Yunan Province of China, known for its traditional consumption of raw mutton and goat milk, also exhibited an elevated prevalence of HEV in goats (19). The results of the research indicated that 74.04% (40/54) and 60% (12/20) of faecal samples tested positive for HEV-RNA. The observed values exhibited a notable increase compared to the values reported in the current study. Further investigation is required to determine whether domestic ruminants can serve as a spillover or genuine reservoir of HEV. This can be achieved through comprehensive observational studies that examine sera and faeces, as well as other tissue samples such as liver, muscle, and spleen.

In the present study, HEV seropositivity in dog is higher than dogs found in Bulgaria (21.1%) (20). This study indicated moderate seropositivity which is lower than what has been reported in previous European studies (13). The HEV prevalence in dogs could be due to feeding dogs with kitchen waste or infected animal offal which has been linked to HEV risk factor in HEV transmission practice.

The study found that overall antibody prevalence was highest in animals aged > 24 months, followed by >18 - ≤24, >6 - ≤12 and >12 - ≤18, and least in 0- ≤6 months. This study contradicts (8), which found that animals under 1-year-old had the highest seroprevalence (21.3%), while those over 2 years old had the least (7.7%). Contrary to Wang et al. (21), piglets showed a 20% higher positive rate than adult pigs, suggesting early infection. Age has been linked to HEV prevalence in some areas (22). However, this association may not be persistent across varied populations (23).

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In this study, HEV prevalence in female was higher than male, inconsistent with study conducted in human in Indonesia where there are high HEV prevalence among females (55 %) than males (47%). This study is nearly similar to report by T (24) where there is high HEV prevalence rate in females (54.8%) than the males (45.2%). Multiple factors, including differences in management practices, may be at play here, suggesting an explanation for the disparity.

Among the pregnant domesticated Animals in the Study Area, Goats exhibited a significantly high HEV prevalence. Though the remaining pregnant domesticated animals were also exhibited HEV prevalence. The presence of HEV prevalence in pregnant domesticated animals in the study locations may be due to the immunosuppressed state experienced during pregnancy and lack of safe drinking water and unhygienic environment.

All the domesticated animals sampled in the study areas where the risk factors of HEV are more common showed HEV high prevalence. In the study locations, the use of unclean water or streams as a source of water supply was identified as a significant risk factor for HEV seropositivity in sheep, dogs, and goats, and proportionately higher in pigs. This is consistent with the findings in other studies in Spain (25) and Turkey (26). Insufficient drinking water treatment and low standards of sanitation in the study areas may be linked to the major cause of high HEV prevalence because contamination of drinking water with animal or human faeces is common in this area. The frequency of HEV was shown to be higher among the majority of domesticated animals that were kept in extended and mixed management practices including those allowed to wander in a contaminated environment. This is as a result of the animal unrestricted availability to contaminated feed and water.

4. CONCLUSION

This study demonstrates that some of the domesticated animal species sampled in Ekiti State, Nigeria, exhibited seropositivity to Hepatitis E virus (HEV). Importantly, strategies are needed to prevent the potential zoonotic transmission, especially among individuals with direct contact with animals. The risk factors such as contaminated environment, unclean water, and the implementation of extensive and mixed animal management practices can increase the susceptibility of animal handlers and domesticated animals to HEV.

CONSENT (WHERE EVER APPLICABLE)

Authors may use the following wordings for this section: "All authors declare that 'written informed consent was obtained from the animal owners (or other approved parties) for publication of this research report. A copy of the written consent is available for review by the Editorial office/Chief Editor/Editorial Board members of this journal."

ETHICAL APPROVAL (WHERE EVER APPLICABLE)

All authors hereby declare that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the appropriate ethics committee"

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Page 3: [1] Comment [B36] **A** **6/16/2024 10:34:00 AM**

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Page 12: [2] Comment [B102] **A** **6/16/2024 2:10:00 PM**

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Page 12: [3] Comment [B104] **A** **6/16/2024 2:10:00 PM**

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Page 12: [4] Comment [B124] **A** **6/16/2024 2:16:00 PM**

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Page 12: [5] Comment [B131] **A** **6/16/2024 2:17:00 PM**

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Page 12: [6] Comment [B147] **A** **6/16/2024 2:22:00 PM**

dogs, cats, horses, cattle, sheep, and goats