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## Short Communication

## Prevalence of Hepatitis E Virus (HEV) in Chicken Droppings in Selected Commercial Poultry Farms in Seven Local Government Areas in Imo State, Nigeria -

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## Abstract

**Background:** Hepatitis E virus causes hepatitis-splenomegaly syndrome or big liver and spleen disease in poultry. It is one of the neglected zoonotic diseases of poultry causing mild clinical symptoms of egg production loss and hepatitis.

**Aim:** The aim of the current study was to determine the prevalence of hepatitis E virus antigen in chicken droppings in seven local government areas in Imo State, Nigeria.

**Materials and Methods:** One hundred and seventy seven (177) chicken droppings were collected from selected poultry farms in seven LGAs in Imo State. The chicken droppings were screened for the presence of Hepatitis E Virus antigen using Anti-HEV ELISA kit (Dia. Pro Diagnostic Bioprobes, Italy).

**Results:** Results showed that 2(6.7%) poultry farms located in Ohaji/Egbema produced positive results. Among all the chicken types sampled, only 2(3.3%) egg-laying broiler breeders were observed to be positive for the virus.

**Conclusion:** The emergence of HEV in commercial poultry farms in Imo State is an unavoidable sign of impending economic losses in poultry production with increased risk of zoonosis in the state.

**Keywords:** Hepatitis splenomegaly syndrome; Chicken droppings; Egg production hepatitis E antigen

## INTRODUCTION

Hepatitis E is a liver disease caused by Hepatitis E Virus (HEV). It is one of the viral hepatitis agents that causes inflammation of the liver, mostly transmitted through water contaminated with faecal material. It can also be transmitted through consumption of uncooked or undercooked meat [1].

The virus is distributed worldwide but the disease is more prevalent in East and South Asia. There is an estimated 20 million infections with hepatitis E annually, leading to about 3.3% mortality [1]. It is an acute, self-limiting infection but can also result in chronic infection with extra-hepatic manifestations, as well as neurological and renal problems. In developing countries, the most prevalent genotypes are 1 and 2, mostly found in humans. Transmission is mostly faeco-oral although transfusion-related infection has been reported [2].

HEV is a positive sense, single-stranded, non-enveloped, RNA icosahedral virus, of the family *Hepeviridae*. The virus has a diameter of about 34 nanometers and RNA genome of approximately 7.5 kilobase pairs in length. At least four HEV genotypes have been identified phylogenetically based on full genome sequencing. These include HEV 1, HEV 2, HEV 3 and HEV4. Genotypes 1 and 2 are known to be human viruses, while genotypes 3 and 4 are zoonotic [3]. Genotypes 3 and 4 have been isolated from humans, pigs, deer and wild boars [1,4].

The genome consists of three open reading frames (ORFs), 5' and 3' non-translated regions, with the largest of the ORFs as ORF1, encoding non-structural proteins; ORF2 encoding putative capsid protein while the small ORF3 encodes a cytoskeleton and overlaps ORF1 and ORF2 partially [5].

In poultry, HEV RNA has been detected in serum, as well as in liver, bile and in oro-nasal secretions with atypical pathologic lesions in affected organs especially liver and spleen. Avian HEV is typically associated with hepatitis-splenomegaly syndrome, however most infected birds can be subclinical. In most cases, infections with HEV in chicken results in drop in egg production with increased mortality of about 1% per week, leading to huge economic losses [6].

Antigenic and genetic closeness of avian HEV and human HEV has been demonstrated [7]. HEV is zoonotic and in healthy humans the infection can be self-limiting, but with severe disease outcome in immunocompromised individuals.

Although information about HEV infection is sketchy in Nigeria, cases have been reported among healthcare workers [8], animal handlers, rural dwellers, and pregnant women [9], and as co-infection in HIV positive patients [10].

A few animals have been reported to harbour HEV. They include poultry birds, pigs, wild boar, and silka deer [1]. However, there is paucity of data on avian HEV (aHEV) antibody studies in Nigeria. In view of this therefore, this study was conducted to determine the prevalence of HEV in chicken droppings in selected commercial poultry farms in seven LGAs in Imo State.

## MATERIALS AND METHODS

### Study Design/Population

The study population included broilers, cockerels, native fowls, old layers and day old chicks selected at random from different commercial poultry farms in Imo State, namely Ahiazu Mbaize, Mbaitoli, Okigwe, Orlu, Ohaji, Owerri North and Owerri West. The specimen size was determined using the formula:

$$n = Z^2 pq$$

$$d^2$$

Where  $n$  = sample size,  $z$  = standard deviation (1.96),  $p$  = prevalence,  $q$  =  $1-p$  and  $d$  = degrees of freedom (0.05). The least number of samples to be collected for the study was calculated to be 150; hence 177 samples were collected.

This study was an ELISA test carried out using HEV Ab version ULTRA kit, from Dia. Pro Diagnostic Bioprobes, Italy. The microplate was already coated with the antibody and the antigens were to be introduced from the chicken droppings. A positive reaction will be shown by a colour change indicating a reaction between the incoming antigen and the antibody already in the microplates.

### Ethical consideration

Ethical clearance was obtained from the Medical Ethical and Scientific Research Committee of the School of Biological Sciences, Federal University of Technology, Owerri, before the commencement of study.

### Inclusion and exclusion criteria

Healthy poultry birds of all ages and breeds were chosen for the



study. Other birds such as turkeys and ducks that were not regularly eaten were excluded from the study population.

### Sample collection

A total of 5 g each of 177 samples each were collected at random from broilers (60), cockerels (23), old layers (50) and day old chicks (44) in the selected LGAs of Imo State, Nigeria. The samples were collected into clean containers, covered tightly, and transported immediately to the laboratory for sampling and analysis.

### Sample preparation

Five grams (5 g) of chicken droppings from the different categories of the poultry birds were dissolved in 10 mL of phosphate Buffered Saline (PBS). The dissolved samples were centrifuged for 2 minutes at 2000 rpm. Hundred microliter (100  $\mu$ L) of the supernatants were collected and introduced into clean test tubes and stored at 4°C till further use.

### Antigen detection

The chicken droppings were screened for the presence of Hepatitis E Virus antigen using Anti-HEV ELISA kit, from Dia. Pro Diagnostic Bioprobes, Italy. The test was carried out based on the manufacturer's instructions. The results were interpreted as described by the manufacturers' kit insert.

Ratio < 0.9: was considered negative

Ratio  $\geq$  0.9 to < 1.1: was considered borderline

Ratio  $\geq$  1.1: was considered positive

### Statistical analyses

Data were assessed by one-way analysis of variance (ANOVA) followed by Dunnett's multiple comparison, Turkey's multiple comparison and student's t-test. All statistical analysis were performed at the  $p < 0.05$  level of significance. All the statistical analysis were done using Graph Pad software version 5.01

## RESULTS

Chicken droppings from seven LGAs in Imo State were samples for HEV virus zoonotic infection. Result obtained showed that 2(6.7%) from Ohaji/Egbema LGA tested positive. This is shown in table 1. In table 2, it was shown that four categories of fowls were screened, namely: day old, old layers, broilers and cockerels with the only positive results in Ohaji/Egbema observed among broilers (3.3%).

**Table 1.** Prevalence of HEV on chicken droppings in selected poultry farms in Imo State.

Poultry Location	No of sample (%)	No +ve (%)	No -ve
Ahiazu	24 (13.6)	0	24
Mbaitoli	25 (14.1)	0	25
Okigwe	20 (11.3)	0	20
Orlu	28 (15.8)	0	28
Ohaji/Egbema	30 (16.9)	2 (6.7)	28
Owerri North	24 (13.6)	0	24
Owerri West	26 (14.7)	0	26
Total	177	2	175

**Table 2.** Types of chicken screened for the hepatitis E virus (HEV) infection in poultry farms in Imo State.

Chicken Type	No of Samples (%)	No +ve (%)	No -ve
	(n = 177)	(n = 2)	(n = 175)
Day old	44 (24.9)	0	44
Old layer	50 (28.2)	0	50
Broiler	60 (33.9)	2 (3.3)	58
Cockerel	23 (13.0)	0	23
Total	177	2	175

## DISCUSSION

Hepatitis E is an enterically transmitted, self-limiting, acute viral hepatitis. It is an ecologically dependent disease of major public health concern especially in resource-poor countries [11]. In this study to determine the prevalence of hepatitis E in poultry farms in LGAs in Imo State, Nigeria, it was observed that only Ohaji/Egbema produced positive results. This may be attributed to the sanitary conditions of the farm of which the ones at Ohaji/Egbema where the positive results were recorded were the least hygienic. According to Khuroo and Khuroo, [12], HEV is a hyperendemic disease of low resource countries with poor sanitation through fecal contamination of food and water supplies. This corroborates the findings of this study.

Among all the chicken sampled, only broiler breeders produced positive results. This may have been due to the fact that these category of chicken are more prone to infection with the virus than other chicken types. This study confirms earlier findings that avian hepatitis-E-virus infection is a disease of broiler breeders and hen-egg layers where it is associated with low mortality and mild decrease in egg production [13,14]. Broiler breeders may have been more infected with HEV in this study than other chicken types probably because of the type of chicken feed they are fed to induce egg production, which may have been contaminated during production by the virus.

Hepatitis E virus infection may not be said to be a major disease of poultry farms in Imo State, since among all the seven LGAs sampled, antibodies were detected in only 6.7% of the farms. In addition, this study revealed that only 3.3% IgG antibodies were detected among the chicken types studied. This is far less than the 44% detected in examined chicken in Vietnam [15], the 20% detected in Brazil [16], the 71% detected in the USA [17], and the 35.09% in China [18]. The poultry farmers in Imo State confirmed that they maintain a high standard hygiene by engaging in adequate and regular clean up exercises. This may probably have accounted for the low rate of infection recorded in this study.

HEV is associated with hepatitis splenomegaly syndrome or big liver and spleen disease in chickens, although the majority of the infected birds are subclinical [19]. Similarly, HEV can lead to severe hepatic disease in humans, more often with higher case fatality rates than either hepatitis A or hepatitis B virus infection [20]. However, avian HEV has been reported not to be zoonotic and does not infect man or other animals [21]. The major impact of the disease is only felt in the poultry industry where it causes major economic losses [14].

listed some of the commonest diseases that affect poultry in Nigeria to include Newcastle (ND), fowl pox, coccidiosis, external and internal parasites, Chronic Respiratory Diseases (CRD), mycoplasmosis, pullorum, tuberculosis, pneumonia, dysentery,



typhoid, aspergillosis and nutritional deficiencies without any reference to avian hepatitis E. Avian hepatitis could therefore be said to be a neglected poultry disease in Nigeria [21].

## CONCLUSION

The present study has revealed that avian hepatitis E is prevalent in Nigeria even though at minimal level in comparison to other parts of the world. Efforts should therefore be geared towards making this viral infection remain at this low level since it has become obvious that the poultry industry is the highest and fastest growing livestock sub-sector in Nigeria, making chickens a readily available source of harvestable lean animal protein to rural/urban households.

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