

Seroprevalence of Hepatitis E Virus Among Patients Attending Federal Medical Centre, Mubi, Adamawa State, Nigeria.

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DOI: <https://doi.org/10.51583/IJLTEMAS.2026.150400033>

Received: 17 October 2025; Accepted: 23 October 2025; Published: 04 May 2026

ABSTRACT

Hepatitis E virus (HEV) is an emerging cause of acute viral hepatitis, particularly in regions with poor sanitation. This study assessed the seroprevalence of HEV among patients attending the Federal Medical Centre (FMC), Mubi, Nigeria. **Methods:** A cross-sectional study involving 100 consenting participants was conducted. Blood samples were analyzed for HEV antibodies using standard serological methods. Demographic data and HEV seroprevalence were analyzed and presented in tables and figures. **Results:** Out of the 100 participants, 28 (28%) were male and 72 (72%) female, with a mean age of 36.40 ± 13.20 years. Overall, 6 (6%) tested positive for HEV antibodies, while 94 (94%) were negative. HEV seroprevalence among females was 4 (83%) and 1 (17%) among males. However, the difference was not statistically significant ($p = 0.98$). The majority of participants were HEV-negative, suggesting low-level circulation of the virus in this population. **Conclusion:** The study found a moderate HEV seroprevalence (6%) among patients at FMC, Mubi. These findings highlight the need for improved hygiene practices and routine screening for HEV, especially in healthcare settings. Further research is recommended to better understand HEV epidemiology in Nigeria.

Keywords: Hepatitis E virus, Seroprevalence, Federal Medical Centre Mubi, Viral hepatitis, Public health, Nigeria.

INTRODUCTION

Hepatitis E virus (HEV) has emerged as a threat to public health, with an estimated number of 70,000 deaths from genotypes 1 and 2 globally. HEV is a quasi-enveloped, single-stranded, positive-sense RNA virus with a genome size between 6.4 and 7.2 kb. Classified into the *Hepeviridae* family *Orthohepevirus A*, HEV has eight known genotypes of which four are pathogenic to humans (HEV-genotypes 1–4); however, the HEV-genotype 7 initially found in dromedary could be also detected in immunosuppressed individuals. The principal mode of transmission in humans is the fecal-oral route, (Mushahwar, 2008). HEV may also be transmitted parenterally, and direct person-to-person transmission is rare. In industrialized countries, the frequency of IgG antibodies to HEV (anti-HEV IgG) varies from 1 to 20%, (Aggarwal and Jameel, 2022), meanwhile in developing countries with poor hygienic conditions, the frequency of anti-HEV IgG varies from 4 to 84.3%. Immunological changes during pregnancy favor the preservation of the foetus in the maternal environment by the suppression of T lymphocyte-induced immunity, thus rendering the pregnant woman more vulnerable to contract viral infections like the HEV, (Navaneethan *et al.*, 2008). HEV infection during the third trimester of pregnancy is associated to a more severe infection that could lead to hepatic failure and possibly maternal death. Clinical features of hepatitis E are indistinguishable from acute hepatitis caused by other hepatotropic viruses. The incubation period ranges from 15–60 days, with a mean of 40 days. HEV-infected persons exhibit a wide clinical spectrum, ranging from asymptomatic infection through acute icteric hepatitis to fulminant hepatitis. The ratio of symptomatic to asymptomatic infection has not been reliably determined, and may vary with viral genotype and epidemiologic setting. Acute hepatitis E usually manifests with icterus, malaise, anorexia, fever,

hepatomegaly, and occasionally pruritus. Studies in non-human primates have shown a relationship between the host's immunological response and degree of liver injury with the dose of viral inoculum. Immunosuppressed persons, in particular solid organ transplant recipients on immunosuppressive drugs, fail to clear the virus leading to chronic HEV infection (lasting >6 months); such cases have mostly had HEV genotype 3 infection, except for one child who had infection with genotype 4 HEV. The laboratory abnormalities in acute hepatitis E are similar to those in acute viral hepatitis caused by other viruses. Laboratory diagnosis of recent HEV infection is based on detection of HEV-specific IgM (IgA in some countries) antibodies or detection of HEV RNA in clinical samples. Past HEV infection is characterized by specific IgG antibodies against ORF2, which may confer protection against reinfection; however, the protective titer and the duration of their persistence are uncertain. Certain population sub-groups are at a higher risk for severe disease following HEV infection. These include pregnant women, persons with pre-existing liver disease and persons with immunosuppression, (Kumar, 2022).

During HEV epidemics, fulminant hepatitis occurs with a disproportionately high rate among pregnant women. Treatment for acute hepatitis E is generally supportive. Chronic hepatitis E in solid organ transplant (SOT) recipients on immunosuppressive treatment has been successfully treated by withdrawal or reduction of immunosuppressive drugs, administration of ribavirin, administration of interferon or a combination of these measures, (Kamar, 2008). Surveillance for hepatitis E disease is very limited and information on disease occurrence and distribution are available only from a few European countries, and most of the data from other parts of the world are limited to reports of outbreaks and case series. By contrast, much more information is available on the seroprevalence of antibodies to HEV, a marker of previous exposure to HEV. However, the interpretation of seroprevalence data is immensely challenging for several reasons. These challenges include the lack of comparability of results from the different assays, high seroprevalence in populations where disease is rare or never reported, the presence of multiple genotypes with different disease patterns and inability of serological tests to distinguish between genotypes, and lack of data for reliable mathematical modelling to determine disease burden from seroprevalence. Furthermore, the majority of seroprevalence studies do not involve a representative sample of any population making it difficult to infer prevalence and trends to the population. Poor laboratory assay performance is the major challenge in interpreting seroprevalence study results. Many studies have shown poor concordance between commercial IgG HEV assays; some reports showed significant batch to batch variability of IgG anti HEV assays, (Abravanel, 2020). The lack of a gold standard test to determine the performance of IgG assays is another challenge. Recent studies comparing the diagnostic accuracy of assays commonly used in Europe and the US for the detection of antibodies against HEV have yielded a significant discrepancy in performance, (Abravanel, 2020). The protective efficacy and the long-term persistence of IgG antibodies against HEV following natural infection has not been clearly determined. The fact that the prevalence of anti-HEV in the population does not reach the very high levels observed with hepatitis A and that attack rates are higher among young to middle aged adults suggests that infections may not confer lifetime protection or infections usually occur later in life.

This intriguing finding is complicated by the recurrence of outbreaks in countries where past epidemics in the population would have resulted in immunity to prevent future outbreaks. The duration of anti-HEV IgG and the protective efficacy of naturally acquired antibodies are important because of the implications for long term vaccine efficacy. In spite of all these challenges, seroprevalence data provides a general picture as to whether HEV infection is endemic in a country, if population has a disproportionately high rate of infection (e.g., persons with animal contact), and for estimation of population level susceptibility to HEV infection. The result obtained could be due to the fact that during an HEV infection IgM type antibodies are synthesized 1 to 4 weeks before clinical manifestations and disappear 8 to 12 weeks after infection. Anti-HEV IgM is the serologic marker of choice for diagnosis of acute HEV infection. On the other hand, anti-HEV IgG antibodies usually persist for many years after infection. HEV-genotypes 1 and 2 (HEV-1 and -2) are transmitted via the fecal-oral route and exclusively infect humans. Infections with HEV-1 can be more severe in HIV, where case fatality rates up to 25% have been reported. (<https://www.who.int/immunization/sage/meetings/2023/o> (2020). Also, certain population groups like immunosuppressed persons and individuals with pre-existing liver disease are more vulnerable to infections with HEV. HEV genotypes 3 and 4, on the other hand, have been found in both humans and animals and are transmitted zoonotic ally via the ingestion of raw or undercooked meat and close contact to infected animals. Furthermore, HEV-3 and -4 can lead to chronic infections, especially in immunocompromised individuals. In patients with chronic HBV infection, super-infection with HEV is a

common cause of liver failure, accounting for 20% of cases in regions endemic for HEV. Furthermore, in some studies a higher HEV seroprevalence in individuals with HBV-related liver disease in comparison to healthy individuals has been observed. In Nigeria, only a few HEV seroprevalence studies have been performed until today which detected a seroprevalence (IgG and total antibodies) between 7.0%–66.7percent in different populations, (Osundare, *et al.*, 2020). In one study, the prevalence of HBV/HEV coinfection was analyzed in Nigerian healthcare workers, reporting a coinfection rate of 27.3%.

Statement of Problem

There is a paucity of information on HEV infection among HIV patients in Federal Medical Centre, Mubi, Adamawa State, Nigeria. In addition, Hepatitis E virus (HEV) is an emerging infectious agent More than 20 million cases of HEV infection occur that causes acute viral hepatitis worldwide. Annually all over the world, with about 70,000 deaths. HEV is the leading cause of enteric ally-transmitted viral hepatitis. Hepatitis E as sporadic disease or outbreaks have occurred in at least sixty-three countries; about half of these countries have reported large outbreaks.

Aims and Objectives of Study

The main aim of this study was to determine the Seroprevalence of Hepatitis E virus among patients attending Federal Medical Centre, Mubi, Adamawa state, Nigeria.

Objectives of the Study

The objectives of this study are to:

- i. determine the seroprevalence of Hepatitis E virus among patients attending federal medical centre, mubi.
- ii. determine the demographic distribution of Hepatitis E virus base on gender of patients attending federal medical centre, mubi.

MATERIALS AND METHODS

Study Area

Federal Medical Centre, Mubi is located in the heart of Mubi town, in the Mubi North Local Government Area of Adamawa State, Nigeria. The hospital is situated along the Mubi-Yola road, approximately 2 kilometers from the town center. Mubi is situated in the northern part of Adamawa State, Nigeria. The town lies between latitudes 10°15'N and 10°20'N, and longitudes 13°15'E and 13°20'E. Mubi has a semi-arid climate with a rainy season that lasts from May to October. The average annual rainfall is around 800 mm, with a mean temperature of 28°C. The vegetation in Mubi is mainly savanna grassland with scattered trees, including acacia and bauna. Mubi is situated in a valley surrounded by hills.

The town has a gentle slope, with an average elevation of 500 meters above sea level. The demographic characteristics of Mubi include: Population: As of 2016, the estimated population of Mubi North Local Government Area was approximately 177,000 people. The age distribution of Mubi is characterized by a high proportion of young people, with over 50% of the population under the age of 20. The sex ratio in Mubi is approximately 1:1, with a slight preponderance of females.

Mubi is inhabited by several ethnic groups, including the Fulani, Hausa, Gudei and Marghi. Federal Medical Centre, Mubi is the main healthcare facility in the town, providing a range of medical services. The hospital provides outpatient services, including general consultations, laboratory tests, and pharmacy services. The hospital has an inpatient ward, providing care for patients requiring hospitalization. The hospital has a maternity ward, providing antenatal, delivery, and postnatal care. The hospital has a laboratory, providing a range of diagnostic tests, including blood tests, urine tests, and stool tests.

Study Population

The study population for this research included the patients attended Federal Medical Centre, Mubi.

Sample Collection

Approximately 4ml of blood was aseptically collected from each volunteer using a sterile syringe and needle from a prominent vein situated at the antecubital fossa of each individual and placed in 5ml plain bottle. Blood samples were transported in insulated containers containing ice packs to the Microbiology laboratory, FMC, Mubi, where the serum of each sample was separated from the whole blood and placed in cryo-vial on the deep freezer for preservation until tested.

Laboratory Analysis for hev Serological Test

The test was performed based on the principle of immunochromatography *invitro* for qualitative determination of HEV antigens in blood (Bio tracer's TM). The tested HEV samples were specific monoclonal antibodies coated on the membrane of the test device. The tested device (cassette) was then taken out of the foil pouch and placed on a clean and placed on a flat surface bench. Then the dispenser cap of the sample tube was twisted off and by holding the tube vertically, five (4) drops of the mixture of the stool sample and buffer were dispensed into the sample well of the cassette test device. The results were recorded after 15 minutes.

Materials Provided with the Test Kits

The testing device (cassette) packed in foil pouch, 1×3ml vial of sample diluents followed the instructions for used.

Materials

Procedure (Rapid Screen Test Using Test Device)

Procedure for Whole Blood Samples

Bring the specimen and test components to room temperature

- The seal was open by tearing along the notch, and the test device was removed from the pouch.
- 1 drop of whole blood was made in 1-2 drops of buffer was added to the sample well
- Results were read within for 10-15 minutes.
- It was ensured that the results were after 15 minutes to avoid error occurrence.

Procedure for Serum/Plasma Samples

- The pouch was opened and pipetted 10µl of serum or plasma into the sample well
- Immediately 2 drops of the Sample was diluted
- The sample was dropped followed by buffer solution into the area while the results were appropriately read within 15 minutes.
- The test device (cassette) was placed on flat surface and the results were read within 15 minutes.

A positive test line appeared after 15 minutes and this is a False/Positive Result. Do not read the results after 15 minutes.

Interpretation of the Test Result

The test results was read and interpreted within 15 minutes.

A red color band on test (T) and control (C) window, showed positive results (antigen detected) was indicated of HEV antigen, in a negative result, a band appeared on the control (C) zone only, (antigen not detected).

If no (C) line is developed the assay is invalid regardless of color development on the (T) line or a total absence of color in both region is an indication of procedure error and or the test reagent has been deteriorated.

Inclusion Criteria

All patients who are willing to give consent/assent

Exclusion Criteria

Patients vaccinated against HEV

Ethical Consideration

The ethical clearance for the study was obtained from the department of microbiology, Adamawa State University, Mubi. Nigeria.

Sample Size Determination

The minimum sample size was calculated from a standard formula for calculation of minimum sample size, (Navaneethan *et al.*, 2008). The formula is as shown below;

$$n = \frac{(z_{1-a})^2(p)(1-p)}{d^2}$$

Where:

n = minimum sample size

Z_{1-a} = the value of standard normal deviation which is at 95% confidence intervals has been found to be 1.96

p = the estimate of the people prevalence obtained from literature review

d = the difference between the true population and the sample that can be tolerated, that is the absolute precision required (in percentage point) on either side of the population.

At prevalence rate of 7%, (Osundare, *et al.*, 2020). Using 5% precision at 95% confidence level, the minimum sample size n for this study was calculated as follows: DATA:

$$Z_{1-a} = 1.96$$

$$p = 7\% = 0.07$$

$$d = 5.0\% = 0.05$$

Therefore;

$$\frac{(1.96)^2 * (0.07) * (1-0.07)}{(0.05)^2}$$

$$= 0.2499$$

$$0.0025$$

$$=99.9$$

$$n=100$$

Minimum sample size was 100.

Study Subjects

A total of 100 subjects was recruited for this study.

Study Design

A hospital-based cross-sectional study design

Study Limitations

This study was limited to adult HIV patients only.

RESULTS

One hundred (100) subjects whose informed consent has been obtained were recruited into the study. The study accessed the Seroprevalence of hepatitis E virus among patients attending Federal Medical Centre, Mubi. Table 4.2 shows a demographic data of the study subjects. It shows that 28(28%) males and 72(72%) females with a mean age of 36.40 ± 13.20 years participated in the study. The tables also revealed the distribution of hepatitis E Virus among gender. It shows that 68(71%) females were hepatitis E negative while 27(29%) males were hepatitis E negative and 4(83%) females were hepatitis E positive while only 1(17%) male was hepatitis E positive as shown in Fig 4.2. It shows a non-significant difference ($p=0.98$).

The frequency of hepatitis E virus among study subjects. It shows that 94(94%) were hepatitis E negative while 6(6%) were hepatitis E positive as shown in Fig 4.1.

Table 4.1: According to Age Distribution of HEV

Age Group (Years)	Total Participants (n)	HEV Positive (n)	HEV Negative (n)	Percentage Positive (%)
10 – 19	10	1 (age 19)	9	10%
20 – 29	20	1 (age 25)	19	5%
30 – 39	30	1 (age 38)	29	3.3%
40 – 49	25	2 (ages 41, 44)	23	8%
50 – 59	10	0	10	0%
60 – 65	5	0	5	0%
Total	100	5	95	5%

The age distribution of the study population shows the highest number of participants (30%) were within the 30–39-year age group, followed by 25% in the 40–49-year group. The lowest participation was recorded among the 60–65-year group (5%).

Out of the 100 participants, **5 tested positive for Hepatitis E Virus (HEV)**. These HEV-positive cases were distributed across the younger to middle-age groups as follows:

- One case (20%) occurred in the **10–19** age group (age 19),
- One case (20%) in the **20–29** group (age 25),
- One case (20%) in the **30–39** group (age 38), and
- Two cases (40%) in the **40–49** group (ages 41 and 44).

No HEV-positive cases were recorded in participants aged **50 years and above**.

These findings suggest that **HEV infection was more commonly observed in individuals under the age of 50**, with a notable clustering between the ages of **38 to 44 years**. However, due to the small number of positive cases ($n = 5$), statistical inference about age-specific risk is limited.

Table 4.2 Demographic distribution of Hepatitis E antibody positivity in gender

Gender	No of tested (%)	HEV (%)	
		Positive	Negative
Female	72 (72)	4 (80)	68 (71)
Male	28 (28)	1 (20)	27 (29)
Total	100	5 (100)	95 (10)

Table 4.2 presents the gender-based distribution of Hepatitis E virus (HEV) antibody positivity among 100 individuals tested. Of these, 72% were female and 28% male. Among the 5 individuals who tested positive, 4 (80%) were female, while 1 (20%) was male, indicating a higher prevalence of HEV antibodies in females.

Expected Frequencies (under null hypothesis of independence):

- **Degrees of freedom (df)** = $(2 - 1)(2 - 1) = 1$
- **p-value** ≈ 0.52

Interpretation of Statistical Test

- **Chi-square value** (χ^2) = 0.4064
- **Degrees of freedom** = 1
- **p-value** ≈ 0.52

Since $p > 0.05$, we **fail to reject the null hypothesis**, indicating that:

There is no statistically significant association between gender and HEV seropositivity in this study population.

- Female, Positive = $(72 \times 6) / 100 = 4.32$
- Male, Positive = $(28 \times 6) / 100 = 1.68$

- **Mean age:** 36.40 years

- **Standard deviation (SD):** ± 13.20 years

Fig 4.1: Frequency of Gender among Study Subjects

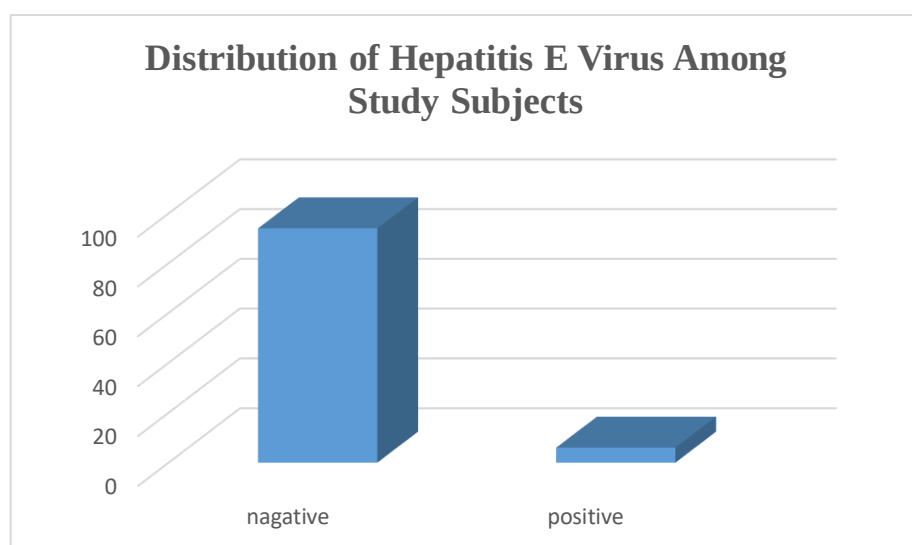
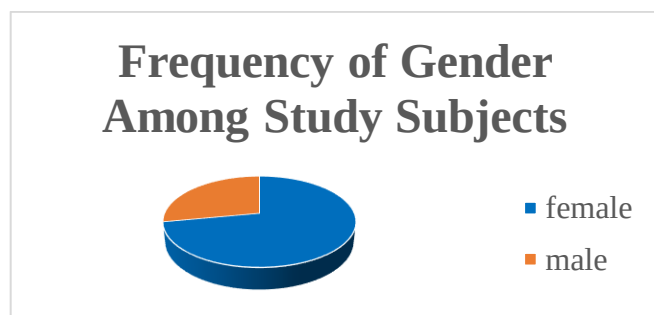


Fig 4.2: Distribution of Hepatitis E Virus Among Study Subjects

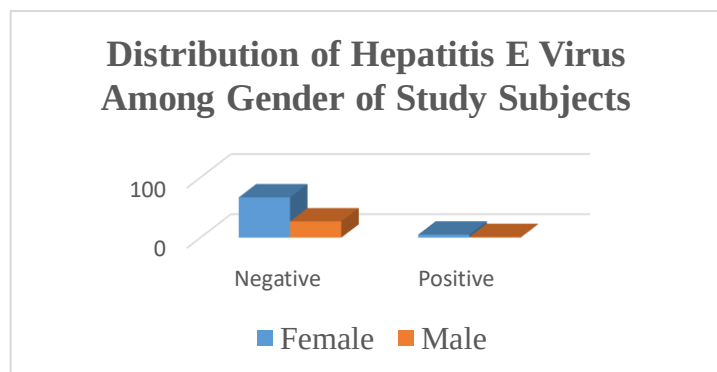


Fig 4.3: Distribution of Hepatitis E Virus among Gender of Study Subjects

DISCUSSION

Hepatitis E virus infection recently has been described as an emerging infection among patients with immunosuppressing conditions of such human immunodeficiency infection, (kaba *et al.*, 2022). In this study, out of 100 participants we found a moderate (5%) of HEV among positive individuals attending FMC, Mubi. Although this rate is higher than the previously reported among similar populations in the industrialized countries. However, it's in agreement with the study of who reported a lower significant rates of HEV infection among some population groups HEV endemic areas of Africa and Asia. This study agreed with finding of Omolade *et al.*, (2018) who reported the prevalence of HEV infection found in this study is lower than the previously reported rate of 44% among Health workers in Nigeria. Although the reason for this difference is not clear, it has been suggested that "health care setting is a home for transmission of infection especially where running water and other materials for hand hygiene are lacking". Nosocomial transmission of HEV infection has

also been reported, (kaba *et al.*, 2022). This present study, demonstrated that majority 94(94%) of the study subjects were hepatitis E virus. This study agrees with the report of Omolade *et al.*, (2018) who stated that 18 of the 2406 participant blood samples were reactive for HEV antibody. Viral hepatitis is a major cause of morbidity and mortality globally. Various viral agents including Hepatitis A, B, C, D, E and G are known as causes of liver diseases such as cirrhosis, hepatocellular carcinoma and liver failure. Hepatitis E disease is a self-limited feco-orally transmitted acute viral infection that occurs most frequently in epidemic forms, (Purcell, 2020). The aetiology of the disease is Hepatitis E Virus (HEV), a non-enveloped RNA virus belonging to the genus *hepevirus* of the family *Hepeviridae*. The virus is transmitted from person to person feco-orally, hence its transmission is highly associated with poor hygiene or sanitation, (Vasickova *et al.*, 2007). While some studies have shown that HEV infected individuals are not at higher risk of acquiring infection than the general population, (Robson *et al.*, 2020), they are at higher risk of developing chronic HEV infection. The seroprevalence of HEV infection in the general population in Nigeria has not been determined, however, the results of this study suggest the need to test for HEV infection in individuals for the early diagnosis and proper management since HEV is known to be fulminant in the presence of underlying liver diseases that are common among infected persons, according to Aggarwal and Gandhi, (2020).

CONCLUSION

The results of this study suggest the need to test for HEV infection in individuals for the early diagnosis and proper management since HEV is known to be fulminant in the presence of underlying liver disease that is common among HEV infected persons. In addition, the use of ART may reduce the incidence of HEV infection.

RECOMMENDATION

- Its therefore recommended that Further studies are required to more clearly determine its prevalence and incidence
- Improve the understanding of its natural history and management, since HEV is known to be fulminant in the presence of underlying liver diseases that are common among HEV Government and NGOS should create more awareness in the community, Personal, water and environmental hygiene should be encouraged in the community.
- Information from this study may provide the need to implement surveillance and assess the hepatitis E Burden in Nigeria.

The Author's Contributions

Ijudigal Musa Papka, David N. Bukbuk, and the author's supervision, writing, review, and editing are among their contributions. Each author has reviewed and approved the published version of the work.

Finances: For this effort, no outside money was secured.

Notes: Papka I.M. edited the English text after D.N Bukbuk.

Authored it.

No conflicts of interest have been disclosed by the authors.

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