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Hepatitis E Virus Exposure at the Human–Pig Interface: Seroprevalence Among Pregnant Women and Slaughter Pigs in Tanzania

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ABSTRACT

Background: Hepatitis E virus (HEV) is a major cause of acute viral hepatitis worldwide and can lead to severe complications, particularly among pregnant women. In sub-Saharan Africa, outbreaks occur periodically, yet routine surveillance remain limited. Domestic pigs are known reservoirs of zoonotic HEV genotypes, raising concern about transmission at the human–animal interface. However, evidence on HEV exposure in both humans and pigs in Tanzania is scarce. This study assessed the seroprevalence of HEV among pregnant women and slaughter pigs in resource-limited communities in Tanzania.

Methods: Cross-sectional study was conducted between September 2023 and July 2024 in Dodoma and Morogoro regions. Pregnant women attending antenatal care clinics and pigs presented for slaughter at municipal abattoirs were systematically sampled. Serum samples were tested for anti-HEV IgG antibodies using a commercial enzyme-linked immunosorbent assay (ELISA). Sociodemographic data from women and pig husbandry practices were collected using structured questionnaires. Descriptive statistics were used to estimate seroprevalence with 95% confidence intervals (CIs). Associations between HEV seropositivity and risk factors were assessed using Chi-square or Fisher's exact tests, followed by logistic regression analysis.

Results: A total of 372 pregnant women and 280 pigs were included in the study. HEV seroprevalence was 15.1% (95% CI: 11.8 – 19.0) among women and 32.9% (95% CI: 27.6–38.6) among pigs. Higher seropositivity among women was observed in the 25 to 34-year age group. In pigs, housing systems, water sources, and farm biosecurity practices were associated with HEV infection.

Conclusion: These findings demonstrate concurrent HEV exposure in humans and pigs in central Tanzania. The higher prevalence in pigs supports their role as potential reservoirs, while detection in pregnant women suggests ongoing community transmission. Strengthening surveillance and adopting a One Health approach integrating maternal health services, veterinary monitoring, and improved farm biosecurity are essential to reduce zoonotic transmission risks.

BACKGROUND

Hepatitis E virus (HEV) is an important cause of acute viral hepatitis worldwide and remains a significant but often neglected public health problem. Globally, an estimated 20 million HEV infections occur annually, resulting in approximately 3.3 million symptomatic cases, 70,000 deaths, and about 3,000 stillbirths each year.[1,2] Although HEV infections occur worldwide, the burden is disproportionately higher in low- and middle-income countries (LMICs), where access to safe drinking water and sanitation infrastructure remains limited.[2-4] In such settings, HEV continues to cause both sporadic infections and large outbreaks.

HEV is a non-enveloped, positive-sense single-stranded RNA virus belonging to the family *Hepeviridae*.[2] The virus is genetically diverse and currently classified into at least eight genotypes (HEV-1 to HEV-8) that differ in host range and transmission patterns.[3] Genotypes 1 and 2 infect humans exclusively and are primarily transmitted through faecally contaminated water, frequently causing epidemics in areas with poor sanitation.[5,6] In contrast, genotypes 3 and 4 are zoonotic and infect both humans and several animal species, including domestic pigs, wild boars, and deer.[7] Human infection with these zoonotic genotypes typically occurs through consumption of undercooked pork products, occupational exposure to infected animals, or environmental contamination.[7] Additional HEV variants have also been detected in camels and rabbits, suggesting that the host range of the virus continues to expand.[3]

Clinically, HEV infection ranges from asymptomatic infection to acute hepatitis characterized by fever, fatigue, nausea, abdominal pain, jaundice, and elevated liver enzymes.[2] While most infections are

self-limiting, severe disease may occur in vulnerable populations. Pregnant women are particularly at risk, especially during the third trimester, where infection with HEV genotype 1 has been associated with case fatality rates of 15 to 25% due to fulminant hepatic failure.[5,6] Infection during pregnancy has also been associated with miscarriage, stillbirth, premature delivery, and neonatal death, making HEV an important contributor to maternal and neonatal morbidity in endemic regions.[5]

In sub-Saharan Africa, HEV infection is increasingly recognized as a significant public health concern. A systematic review reported widespread evidence of HEV infection across the continent, with seroprevalence varying considerably between populations and geographic regions.[4] Several outbreaks have been documented in refugee camps, internally displaced populations, and communities with limited access to safe water and sanitation, highlighting the vulnerability of populations living in resource-limited environments.[4,6,7] Although waterborne transmission remains dominant in many settings, increasing evidence suggests that zoonotic transmission through animal reservoirs may also contribute to HEV circulation.

Domestic pigs are important carriers of zoonotic HEV genotypes and represent a potential source of human infection.[7-9] Studies conducted in Uganda and Ghana reported HEV seroprevalence ranging from approximately 10% to more than 40% among pigs, indicating widespread viral circulation in swine populations.[9-11] Humans may become infected through direct contact with pigs, occupational exposure during pig farming or meat processing, or by consuming undercooked pork products.[7,12,13] Consequently, pig farmers, slaughterhouse workers, butchers, and pork consumers may face increased risk of infection. In Tanzania, HEV remains poorly studied and likely underdiagnosed, and pregnant women may be particularly vulnerable because routine HEV screening is not commonly performed.[7,12]

Despite these potential risks, data on HEV exposure among both human and pig populations in Tanzania remain scarce, particularly in communities where close interaction between humans and pigs occurs. Most existing studies in Africa have examined either human populations or animal reservoirs independently, and few investigations have explored HEV circulation simultaneously in humans and pigs within the same communities. Therefore, this study aimed to determine the seroprevalence of HEV among apparently healthy pregnant women and pigs presented for slaughter in resource-limited communities of Dodoma and Morogoro, Tanzania, providing baseline evidence on HEV circulation at the human–pig interface to inform surveillance and One Health prevention strategies.

METHODS

Study Design and Setting

This cross-sectional study was conducted between September 2023 and July 2024 in central Tanzania. Human participants were recruited from antenatal clinics at St. Francis Referral Hospital in Ifakara, Morogoro Region, which serves a large rural and peri-urban population in the Kilombero Valley. Pig samples were collected from municipal slaughter facilities located in Ifakara Town Council (Morogoro Region) and Mpwapwa District (Dodoma Region). These sites were selected because they represent important areas of pig production and pork consumption in central Tanzania and provide an opportunity to explore HEV exposure at the human–pig interface.

Study Population

The study involved two populations: The first population consisted of pregnant women attending antenatal care (ANC) clinics in selected public health facilities in Ifakara, Morogoro region. Eligible participants were pregnant women who were apparently healthy at the time of recruitment, attending routine ANC services, willing to participate, and able to provide written informed consent. Pregnant women presenting with clinical signs suggestive of hepatitis or severe illness were excluded. The second population consisted of pigs presented for slaughter at municipal abattoirs and slaughter slabs in the study regions. Pigs aged two months or older, regardless of sex, breed, or farm of origin, were eligible for inclusion. Animals that were severely ill or unfit for slaughter were excluded.

Sample Size Determination

The sample size for both populations was determined using the single population proportion formula as describe by Cochran,[14] the following statistical parameters were used: Z = standard normal deviate corresponding to a 95% confidence level, because reliable estimates of HEV prevalence in the study populations were limited, a prevalence of 50% ($P = .5$) was assumed to maximize the sample size. A 95% confidence level and a 5% margin of error (d = 0.05) were used. The minimum estimated sample size was therefore 384 participants. During the study period, 372 pregnant women attending ANC clinics were recruited. In addition, 280 pigs presented for slaughter were sampled. These numbers reflected the feasible number of participants and animals available during the study period while still allowing reliable estimation of HEV seroprevalence.

Sampling Procedure

Pregnant women were recruited from selected antenatal care clinics in the study regions. A systematic random sampling approach was applied. Based on the average daily ANC attendance recorded in clinic registers, every fifth eligible pregnant woman attending the clinic was approached for participation. Recruitment continued throughout the study period until the desired sample size was reached. Whereas, pigs were sampled at municipal slaughterhouses and slaughter slabs within the study areas. A systematic random sampling method was used along the slaughter line. Every fifth pig presented for slaughter was selected for sampling. Sampling was conducted across multiple days to ensure that pigs originating from different farms supplying the slaughter facilities were represented.

Data Collection

Data collection was conducted by a team of trained research assistants and clinicians between September 2023 and July 2024. A structured questionnaire was used to collect socio-demographic and obstetric information

from pregnant women. The questionnaire was initially developed in English and then translated into Kiswahili, the national language of Tanzania, to ensure that participants clearly understood the questions. Prior to the main study, the questionnaire was pre-tested among 20 pregnant women attending an ANC clinic in a health facility outside the study sites. The purpose of the pre-test was to evaluate the clarity, relevance, and cultural appropriateness of the questions. Based on feedback from the pre-test, minor modifications were made to improve question wording and the logical flow of the questionnaire.

Interviews were conducted in Kiswahili by trained research assistants with backgrounds in public health or nursing, under the supervision of the principal investigators. Each interview lasted approximately 10–15 minutes and was conducted in a private area within the health facility to ensure confidentiality. The questionnaire captured information on age, parity, pregnancy trimester, residence, occupation, and HIV status (where available from ANC records). As for pigs, a standardized data collection form was used to record relevant animal and farm management characteristics. Information collected included sex, estimated age category, breed, housing conditions, water sources, feeding practices, and selected farm biosecurity measures, such as manure disposal and the presence of footbaths.

Laboratory Procedures

For pregnant women, approximately 5 mL of venous blood was collected by trained clinicians using sterile vacutainer tubes. For pigs, 5–10 mL of blood was collected from the jugular vein immediately after slaughter. All samples were transported to the laboratory in cool boxes with ice packs. Upon arrival, samples were centrifuged at 3,000 rpm for 10 minutes, and serum was separated, aliquoted into cryovials, and stored at −20°C until analysis.

Serological testing for anti-HEV IgG antibodies was performed using a commercial enzyme-linked immunosorbent assay (ELISA) kit (MP Diagnostics HEV ELISA 4.0, USA) according to the manufacturer's instructions. Briefly, serum samples and controls were added to antigen-coated microplate wells and incubated to allow binding of HEV-specific antibodies. After washing, enzyme-conjugated secondary antibodies were added, followed by a substrate solution to produce a measurable color reaction. Optical density was measured using a microplate reader at the recommended wavelength. Results were interpreted using the kit's cut-off values. Samples were classified as reactive (positive), non-reactive (negative), or equivocal, and equivocal samples were retested to confirm the result.

Data Management and Statistical Analysis

Data were double-entered into Microsoft Excel and exported to SPSS version 25 and R statistical software for analysis. Descriptive statistics were summarized as frequencies, proportions, means, and standard deviations. HEV seroprevalence was calculated as the proportion of samples testing positive with 95% confidence intervals (CIs). Associations between HEV seropositivity and explanatory variables were assessed using the Chi-square test or Fisher's exact test where appropriate. Variables with P values < 0.20 in bivariate analysis were included in multivariable logistic regression models to identify independent predictors of HEV infection. Adjusted odds ratios (aORs) with 95% confidence intervals were reported, and *P* values < .05 were considered statistically significant.

Ethics Approval and Consent to Participate

This study was conducted in accordance with the ethical principles outlined in the Declaration of Helsinki. Ethical approval was obtained from the National Institute for Medical Research (NIMR), Tanzania (Reference No. NIMR/HQ/R.8a/Vol.IX/4744). Permission to conduct the study was also obtained from the District Executive Director of Mpwapwa District Council and the Executive Director of Ifakara Town Council, as well as from the management of St. Francis Referral Hospital and St. Francis University College of Health and Allied Sciences (SFUCHAS). Written informed consent was obtained from all participating pregnant women prior to enrolment. Participation was voluntary, and confidentiality of all participants was maintained by using unique identification codes instead of personal identifiers. For animal sampling, approval and permission were obtained from the relevant veterinary authorities, and all procedures were conducted in accordance with Tanzanian animal welfare and biosafety regulations.

RESULTS

Characteristics of Pregnant Women

A total of 372 pregnant women attending antenatal care clinics at St. Francis Referral Hospital in Ifakara, Morogoro Region, were enrolled in the study. The mean age was 27.5 ± 6.4 years (range: 14–44 years). Most women were aged 25–29 years (25.3%) or 20–24 years (24.2%), and over half were in their second trimester (52.2%). The majority were HIV-negative (96.2%) and engaged in informal occupations such as subsistence farming or petty trade.

Characteristics of Pigs

A total of 280 pigs were sampled from slaughter facilities in Ifakara Town Council (Morogoro Region) and Mpwapwa District (Dodoma Region). The sample included 151 males (53.9%) and 129 females (46.1%). Most pigs were crossbreeds (55.4%), followed by exotic (28.6%) and local breeds (16.1%). The majority were kept under closed housing systems (87.5%), while smaller proportions were raised under semi-closed (8.6%) or free-range systems (3.9%).

HEV Seroprevalence among Pregnant Women

Out of the 372 pregnant women, 56 tested positive for anti-HEV IgG antibodies, giving an overall seroprevalence of 15.1% (95% CI: 11.8 – 19.0). HEV seroprevalence varied across age groups. The highest prevalence was observed among women aged 25–29 years (22.3%), followed by those aged 35–39 years (20.0%). Women aged ≤19 years and 20–24 years showed lower prevalence (15.2% and 15.6%, respectively). Seropositivity also varied slightly by pregnancy trimester. Women in the first trimester had a prevalence of 22.1%, compared with 17.5% in the second trimester and 17.3% in the third trimester. However, these differences were not statistically

significant. Among HIV-positive women, 2 of 14 (14.3%) tested positive for HEV antibodies, compared with 66 of 358 (18.4%) among HIV-negative women. The results are presented in Table 1.

Pig population Characteristics and HEV Prevalence

A total of 280 pigs were sampled at municipal slaughter facilities in the study area. The sampled population included 151 males (53.9%) and 129 females (46.1%). Pigs originated from different slaughter locations and were sampled across several months during the study period. Among the 280 pigs sampled, 92 tested positive for anti-HEV IgG antibodies, resulting in an overall seroprevalence of 32.9% (95% CI: 27.6–38.6). HEV seroprevalence differed slightly between sexes. Female pigs showed a higher prevalence (36.4%) compared with males (29.8%), although this difference was not statistically significant. The summary of the results are presented in Table 2.

Comparison of HEV Seroprevalence Between Humans and Pigs

A comparison of HEV seroprevalence between the two study populations revealed that pigs had a markedly higher prevalence (32.9%) than pregnant women (18.3%). This finding suggests that pigs may represent an important reservoir for HEV transmission in the study area. The higher prevalence observed in pigs supports the hypothesis that zoonotic transmission may contribute to human HEV exposure, particularly in communities where pig farming and pork consumption are common.

Risk Factor Assessment

Pregnant women: factors associated with HEV seropositivity, In bivariate analysis, HEV seropositivity varied by age group (highest in 25–29 years: 19.1%) but age was not statistically associated with seropositivity (***P*** = .134). Seroprevalence did not differ significantly by trimester (P =.578) or HIV status (P = .704). In multivariable analysis (age group, trimester, HIV status, occupation), no factor remained independently associated with HEV seropositivity (all adjusted P values > .05). The results are presented in Table 3A.

In pigs factors associated with HEV seropositivity was highest in semi-closed housing (41.7%) compared with closed (32.2%) and free-range systems (27.3%), though the difference was not statistically significant (P = .594). Seroprevalence did not differ significantly by water source (P = .967) or biosecurity category (P = .291). On the other hand breed showed evidence of association in crude analysis: compared with local breeds, crossbreeds had lower odds of HEV seropositivity (crude OR = 0.50, 95% CI = 0.25–0.98, P = .044). After adjustment (housing, water, biosecurity, sex, age category, district), the association attenuated and became borderline (adjusted OR = 0.50, 95% CI = 0.24–1.03, P = .060). District also showed variation (Ifakara TC: 40.0% vs Mpwapwa: 28.2%); however, this was not statistically significant in the adjusted model. The summary of the results are presented in Table 3B.

TABLE 1: Characteristics of Pregnant Women and HEV Seropositivity (n = 372)

Category	Item	N	HEV+ n (%)	*P value*
Age group	≤19	46	7 (15.2)	.134
	20–24	90	10 (11.1)	
	25–29	94	18 (19.1)	
	30–34	78	8 (10.3)	
	35–39	50	8 (16.0)	
	≥40	14	5 (35.7)	
Trimester	First	68	14 (20.6)	.578
	Second	194	29 (14.9)	
	Third	110	13 (11.8)	
HIV status	Negative	358	55 (15.4)	.704
	Positive	14	1 (7.1)	
Occupation	Homemaker	332	49 (14.8)	.898
	Business	29	5 (17.2)	
	Employed/Other	11	2 (18.2)	

TABLE 2: Pig Husbandry Characteristics and HEV Seropositivity (n = 280)

Category	Item description	N	HEV+ n (%)	*P value*
Housing system	Free-range	11	3 (27.3)	.594
	Semi-closed	24	10 (41.7)	

Continue

TABLE 2: Continued

Category	Item description	N	HEV+ n (%)	*P value*
	Closed	245	79 (32.2)	
water source	Borehole	242	80 (33.1)	.967
	River	23	7 (30.4)	
	Others	15	5 (33.3)	
Breed	Local	45	19 (42.2)	.098
	Cross	155	44 (28.4)	
	Exotic	80	29 (36.3)	
Biosecurity	No	181	55 (30.4)	.291
	Yes	99	37 (37.4)	

TABLE 3A: Logistic Regression for Predictors of HEV Seropositivity

Variable	Category	Adjusted OR	95% CI	*P value*
Age group	20–24	0.69	0.24–1.97	.488
	25–29	1.28	0.48–3.39	.616
	30–34	0.62	0.21–1.85	.392
	35–39	1.06	0.35–3.22	.919
	≥40	3.25	0.83–12.79	.091
Trimester	Second	0.7	0.33–1.47	.346
	Third	0.7	0.31–1.60	.402
HIV status	Negative			
	Positive	0.4	0.05–3.18	.385
Occupation	Homemaker			
	Business	1.1	0.38–3.14	.86
	Employed/Other	1.23	0.25–6.08	.8

TABLE 3B: Pigs (Outcome: HEV IgG positive)

Variable	Category	Adjusted OR	95% CI	*P value*
Housing	Free-range			
	Closed	1.25	0.31–5.14	.752
	Semi-closed	2.06	0.41–10.42	.38
Water source	Borehole			
	Others	1.18	0.38–3.68	.771
	River	0.88	0.34–2.31	.798
Breed	Local			
	Cross	0.5	0.24–1.03	.06
	Exotic	0.54	0.22–1.28	.161
Biosecurity (Yes vs No)	Yes	1.49	0.87–2.55	.144
Sex	Male vs Female	0.74	0.44–1.25	.262
Age (months)	Above 10 vs Under10	0.91	0.55–1.52	.725
District	Mpwapwa vs IfakaraTC	0.7	0.39–1.25	.231

DISCUSSION

This study provides new evidence that hepatitis E virus (HEV) is circulating at the human–animal interface in central Tanzania. HEV exposure was detected in both pregnant women and pigs, with a substantially higher seroprevalence observed in pigs. This finding supports previous evidence that domestic pigs are an important reservoir for zoonotic HEV transmission.[7,14,15] Although no strong predictors of infection were identified among pregnant women, the detection of HEV antibodies suggests ongoing but largely unnoticed community transmission. In pigs, housing conditions and farm management practices appeared to influence infection patterns, indicating that livestock production systems may contribute to the maintenance and spread of the virus. These findings highlight the importance of integrated surveillance using a One Health framework.[16-18]

The higher HEV seroprevalence observed in pigs compared with humans in this study is consistent with findings from other regions where zoonotic HEV genotypes circulate in swine populations. Studies conducted in Uganda reported HEV seroprevalence in pigs ranging from approximately 11% to over 40%, indicating widespread exposure among domestic swine populations.[18,19] Similarly, research conducted in Ghana demonstrated active circulation of HEV in pigs and confirmed the presence of zoonotic genotype 3 strains, suggesting that pigs may serve as an important source of human infection.[20-22] In sub-Saharan Africa, HEV epidemiology in humans has largely been documented during outbreak investigations, often associated with contaminated water sources and poor sanitation infrastructure.[5,21,23] Such outbreaks have been reported in several African countries and may lead to significant morbidity and mortality, particularly among pregnant women.[9,24,25]

The seroprevalence observed in this study suggests the presence of endemic transmission rather than an acute outbreak. Silent or under-recognized transmission of HEV may occur in many African settings because routine surveillance is limited and HEV testing is rarely included in the clinical evaluation of hepatitis cases.[5,25] Evidence from Tanzania itself remains limited. Previous studies in the country have largely focused on other viral hepatitis infections such as hepatitis A, B, and C, while HEV has received comparatively little attention. The detection of HEV antibodies in both pregnant women and pigs in the present study therefore contributes important baseline data and supports earlier suggestions that HEV may be circulating in Tanzanian communities but remains underdiagnosed.[26,27]

Zoonotic Risk, Occupational Exposure, and Food Safety

The detection of HEV antibodies in both pigs and humans suggests a potential risk of zoonotic transmission in settings where close interaction between people and livestock occurs. Pig farming has expanded in regions such as Morogoro and Dodoma due to increasing demand for pork products and the economic importance of small-scale livestock production. In such environments, HEV circulating in swine populations may increase the likelihood of spill over infection through environmental contamination, direct contact with infected animals, or exposure during slaughter and meat processing.[7,13,28,29] Individuals who work closely with pigs, this will include farmers, slaughterhouse workers, butchers, and meat vendors may therefore face a higher risk of infection. Previous studies conducted in Europe and Asia have reported higher HEV seroprevalence among swine workers compared with the general population, highlighting occupational exposure as an important pathway of zoonotic transmission.[2,3,30,31] Foodborne transmission is another recognized pathway. Consumption of undercooked or contaminated pork products has been associated with HEV infection in several countries.[2,31,32] Strengthening meat inspection systems, promoting hygienic slaughter practices, and encouraging thorough cooking of pork products are therefore important measures to reduce the risk of HEV transmission.

One Health Approach

The findings of this study reinforce the need for a One Health approach that integrates human health, veterinary health, and environmental surveillance. Effective prevention of HEV transmission requires coordinated efforts across multiple sectors.[30] In the public health sector, increased awareness and improved diagnostic capacity for HEV are needed, particularly in antenatal care settings where pregnant women represent a vulnerable population. In the veterinary sector, improving farm biosecurity, managing animal waste, and strengthening monitoring of pig health could help reduce viral circulation in swine populations. Environmental interventions such as improved sanitation and safe water access are also essential components of HEV prevention strategies.[5,13,32-34]

Strengths and Limitations

A major strength of this study is its integrated human–animal design, which allowed simultaneous investigation of HEV exposure in a vulnerable human population and a potential animal reservoir. This approach provides valuable insight into HEV circulation at the human–animal interface. The use of validated serological assays and standardized data collection procedures enhanced the reliability of the findings. Additionally, information on pig husbandry practices provided insight into farm management factors that may influence HEV transmission in animal populations. However, several limitations should be considered when interpreting these results. First, the cross-sectional design limits the ability to determine the direction or timing of transmission. Second, molecular testing and viral genotyping were not performed, preventing confirmation of whether the same HEV strains circulate between humans and pigs. Third, the human component of the study was limited to pregnant women attending antenatal care at a single hospital, which may limit generalizability to other populations. Finally, environmental sampling and occupational exposure data were not collected, which could have provided additional insight into potential transmission pathways.

Policy and Practice Implications

The detection of HEV antibodies in both pregnant women and pigs highlights the need to strengthen HEV surveillance in Tanzania. Integrating HEV awareness and testing into antenatal care, improving pig farm biosecurity, and promoting safe pork handling are essential. These

actions should be implemented through a coordinated One Health approach linking human, animal, and environmental health sectors.

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