Journal of Zoonotic Diseases
https://jzd.tabrizu.ac.ir/
Published by the University of Tabriz
Online ISSN: 2717-2910

Serological evidence of zoonotic Hepatitis E virus transmission: A cross-sectional analysis of high-risk occupational groups in central Kalimantan, Indonesia

Nawan Nawan[1], Septi Handayani[2], Agnes Immanuela Toemon[3], Seth Miko[4]

[1] Department of Microbiology, Faculty of Medicine, University of Palangka Raya, Palangka Raya, Indonesia
[2] Department of Biochemistry, Faculty of Medicine, University of Palangka Raya, Palangka Raya, Indonesia
[3] Department of Parasitology, Faculty of Medicine, University of Palangka Raya, Palangka Raya, Indonesia
[4] University of Palangka Raya, Palangka Raya, Indonesia

Article type:
Original article

Keywords:
Hepatitis E Virus
Zoonosis
Risk Factors
Occupational
Indonesia

Article history:
Received:
September 27, 2025
Revised:
October 17, 2025
Accepted:
December 12, 2025
Available online:
December 19, 2025

Abstract
Hepatitis E virus (HEV) is a significant cause of acute viral hepatitis, with zoonotic transmission from pigs representing a recognized public health concern. In Central Kalimantan, Indonesia, where close human-pig interaction is common, specific risk factors for HEV exposure remain poorly understood. This knowledge gap hinders the development of effective, targeted public health strategies. This study aimed to identify the occupational and environmental factors linked to HEV seropositivity in this high-risk population (n=59). The cohort was stratified into four exposure groups: pig farm workers (n=14), butchers/pork sellers (n=7), household pig owners (n=28), and a control group with no direct pig contact (n=10). A structured questionnaire gathered detailed data on demographics, occupational history, and hygiene practices. Serum samples were analysed for anti-HEV IgG antibodies using a commercial ELISA. The overall seroprevalence was 8.5% (5/59). All seropositive cases were found exclusively among participants with direct pig exposure, suggesting a zoonotic pathway. A clear risk gradient emerged when comparing the groups: Butchers and pork sellers showed the highest prevalence at 28.6%, followed by pig farm workers at 14.3%. In contrast, risk was substantially lower for individuals raising pigs residentially (3.7%). No infections were detected in the control group (0%). All seropositive individuals reported prolonged pig contact and inconsistent PPE use. In conclusion, our findings are consistent with occupational exposure being a key driver of HEV infection in Palangka Raya. This underscores the pig-to-human transmission route and highlights the need for targeted interventions focusing on hygiene and PPE use for high-risk workers.

Introduction

Hepatitis E virus (HEV), a positive-sense, single-stranded RNA virus of the Hepeviridae family, has emerged as a significant global public health concern, responsible for an estimated 20 million infections annually and over 44,000 deaths (1). While historically associated with large, self-limiting waterborne outbreaks in developing

***Corresponding author:** *nawan@med.upr.ac.id*
https://doi.org/10.22034/jzd.2025.20947
https://jzd.tabrizu.ac.ir/article_20947.html
Cite this article: Nawan N., Handayani S., Toemon A.I., Miko S. Serological evidence of zoonotic Hepatitis E virus transmission: A cross-sectional analysis of high-risk occupational groups in central Kalimantan, Indonesia. Journal of Zoonotic Diseases, 2026, 10 (1): 1092-1098.

nations (genotypes 1 and 2) (2-4), the scientific understanding of HEV has evolved dramatically. It is now recognized as a major zoonotic pathogen, particularly genotypes 3 and 4, for which domestic pigs and wild boars serve as the primary reservoirs (5, 6). In industrialized nations, sporadic, locally acquired infections are increasingly linked to the consumption of contaminated pork products, making HEV a prime example of a "One Health" issue that bridges animal, human, and environmental health (7).

In Southeast Asia, HEV is endemic, with seroprevalence rates varying widely depending on sanitation levels and local dietary customs (8). Indonesia, as an archipelago with diverse cultures and ecosystems, presents a complex epidemiological landscape for HEV. Previous studies have documented outbreaks and established high seroprevalence in regions with significant pig populations, such as Bali and West Kalimantan (9, 10). These reports confirm the circulation of zoonotic HEV genotypes and underscore the pig-to-human transmission axis as a key public health challenge. However, much of this research has focused on establishing baseline prevalence, leaving a critical gap in our understanding of the specific transmission dynamics and risk factors within vulnerable communities.

This risk profile is particularly relevant in Central Kalimantan, a province on the island of Borneo. The region's capital, Palangka Raya, is predominantly inhabited by the Dayak people, for whom pig husbandry and pork consumption are deeply integrated into their cultural and economic life. Pigs are often raised in proximity to human dwellings, and community members are frequently involved in the full spectrum of the pork production chain. This intimate human-animal interface creates a hypothesized high-risk environment for zoonotic HEV transmission (11), yet the specific risk factors within this unique setting remain uncharacterized. Without a clear understanding of which activities pose the greatest threat, public health strategies cannot be effectively targeted.

The urgency of characterizing these risks is amplified by the unique clinical challenges of HEV. Unlike other forms of viral hepatitis, HEV infection is associated with an exceptionally high mortality rate—up to 25%—in pregnant women (12). Furthermore, in immunocompromised individuals, such as organ transplant recipients, HEV can establish a chronic infection, leading to rapid liver fibrosis and cirrhosis (13, 14). Given these severe potential outcomes and the suspected high-risk environment in Palangka Raya, a granular understanding of transmission determinants is imperative. Therefore, this study was designed to identify the specific occupational and environmental risk factors associated with HEV seropositivity in this potential zoonotic hotspot.

Materials and Methods

Study Design and Population

A cross-sectional study was conducted in Palangka Raya, the capital city of Central Kalimantan, Indonesia, a region characterized by close human-pig interaction. A total of 59 adult participants were enrolled after providing written informed consent. To investigate the role of exposure type on HEV risk, the study population was purposefully stratified into four distinct groups. These included two high-intensity occupational groups: pig farm workers (n=14) and butchers/pork sellers (n=7). A third group consisted of household pig owners (n=28), representing individuals with lower-intensity but regular exposure. The fourth group was a control group (n=10) composed of residents who consume pork but reported no occupational or direct household contact with live pigs, serving as a baseline for community-level exposure.

Data and Sample Collection

Following enrolment, a structured questionnaire was administered to each participant by trained field staff. The questionnaire collected comprehensive data on demographics (age, gender, education), occupational history, frequency and nature of animal contact, and personal hygiene practices (e.g., hand washing, use of protective equipment).

Sample collection for this study was conducted in 2012. Immediately after the interview, a 5 mL venous blood sample was collected from each participant by a certified phlebotomist. The samples were processed to separate the serum, which was then aliquoted and stored at -80°C until laboratory analysis.

Serological Analysis

All serum samples were screened for anti-HEV Immunoglobulin G (IgG) antibodies to determine past or ongoing HEV exposure. The analysis was performed using a commercially available enzyme-linked immunosorbent assay (ELISA) kit (VHE ELISA 4.0v; MP Biomedicals Asia Pacific Pte Ltd., Singapore). All procedures were conducted strictly in accordance with the manufacturer's protocol. The optical density (OD) of each well was read using a microplate reader at 450 nm. A sample was considered positive if its OD value exceeded the calculated cut-off value, which was determined based on the OD of the negative controls provided in the kit.

Statistical Analysis

Data were analyzed using descriptive statistics. Seroprevalence was calculated as a percentage. To assess the statistical significance of the difference in seroprevalence between high-risk (occupational) and lower-risk (community) groups, Fisher's Exact Test was performed due to the small sample number. A p-value < 0.05 was considered statistically significant.

Results

Participant Characteristics and Overall Seroprevalence

A total of 59 participants from Palangka Raya were enrolled in this study. Of these, 5 tested positive for anti-HEV IgG antibodies, yielding an overall seroprevalence of 8.5%. Notably, all seropositive cases were exclusively found within the three groups that reported direct and regular contact with pigs, strongly suggesting a zoonotic transmission pathway. No evidence of HEV exposure was detected in the control group.

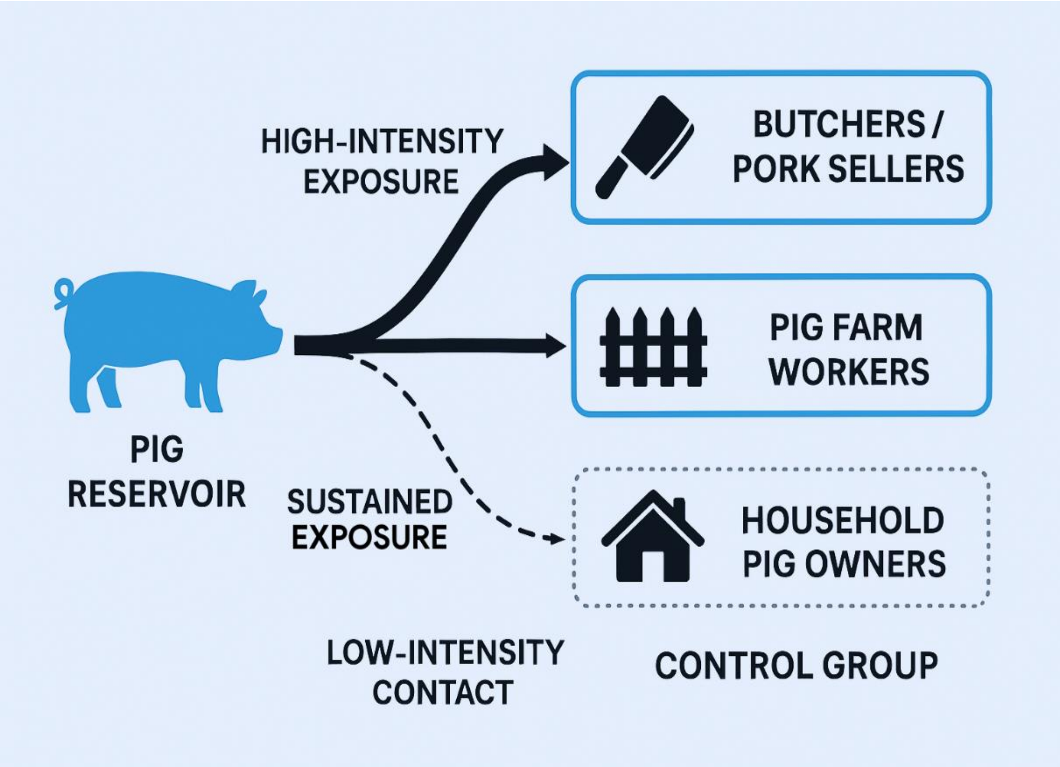

Fig. 1. A schematic of Zoonotic HEV Transmission Routes and Risk Gradient in Central Kalimantan.

The diagram illustrates the transmission from the pig reservoir to different human exposure groups. The thickness of the arrows represents the relative risk, which is highest for occupational groups (butchers and farm workers) due to intense and direct contact with infectious materials, and lowest for community members with no direct contact.

Occupational and Environmental Risk Factors for HEV Seropositivity

A clear risk gradient was observed when seroprevalence was analysed by exposure group, highlighting the critical role of occupational contact in HEV transmission. The results are summarized in Table 1. The highest seroprevalence was found among butchers and pork sellers, with 28.6% (2 out of 7) testing positive. This group, involved in the daily slaughter and handling of raw pork, represented the highest-risk population in our study. Pig farm workers, who engage in intensive, daily management of live animals, also demonstrated a significantly elevated risk, with a seroprevalence of 14.3% (2 out of 14).

In stark contrast, the risk was substantially lower for individuals who raised pigs in a non-commercial, residential setting. Among these household pig owners, the seroprevalence was only 3.7% (1 out of 28). This finding suggests that lower-intensity contact may mitigate transmission risk. As expected, the control group, with no direct contact with pigs, had a seroprevalence of 0.0% (0 out of 10). When combined for statistical analysis, the high-risk occupational groups (butchers and farm workers) had a collective seroprevalence of 19.0% (4/21). In contrast, the lower-risk community groups (household owners and controls) had a seroprevalence of only 2.6% (1/38). This difference was found to be statistically significant (Fisher's Exact Test, $p = 0.048$). From the questionnaire data, all five seropositive individuals reported that their occupational or pig-handling activities had been ongoing for more than one year, and none of the seropositive participants in the high-risk occupational groups reported consistent use of personal protective equipment (PPE).

Table 1. Seroprevalence of Anti-HEV IgG Across Different Exposure Groups

Exposure Group	No. of Participants (n)	No. of Seropositive Cases	Seroprevalence (%)
Butchers / Pork Sellers	7	2	28.6%
Pig Farm Workers	14	2	14.3%
Household Pig Owners	28	1	3.7%
No Direct Pig Contact (Control)	10	0	0.0%
Total	59	5	8.5%

Discussion

This study provides the first detailed risk factor analysis for Hepatitis E virus exposure in Central Kalimantan. Our finding of an overall seroprevalence of 8.5% among the general participants aligns with the broad spectrum of HEV endemicity reported across Southeast Asia. For instance, seroprevalence rates in neighboring regions can range from as low as 4% in some communities to over 20% in others, depending on sanitation and the level of swine contact (8). While our overall rate provides a useful regional benchmark, the more critical insight context-specific insight from our study is that the risk of HEV infection is not uniformly distributed. Instead, it is overwhelmingly dictated by the nature and intensity of occupational exposure to pigs. The clear risk gradient, with the highest prevalence among butchers and farm workers and virtually zero risk in the non-exposed control group, strongly reinforces the role of pigs as the primary zoonotic reservoir in this community and highlights specific professional activities as critical transmission pathways.

The exceptionally high seroprevalence observed in the occupational groups—28.6% among butchers and 14.3% among pig farm workers—points directly to the specific tasks associated with the pork production chain as primary drivers of infection. For butchers, activities such as slaughtering and evisceration create significant opportunities for exposure to infectious materials like blood and organ tissues, particularly the liver, a known site of HEV replication (15-18). For farm workers, daily tasks like cleaning enclosures and managing animal waste involve sustained contact with a contaminated environment. The risk in both settings is likely amplified by the reported lack of consistent PPE use, which allows for direct viral entry through skin abrasions or mucous membranes (19). The stark difference in risk between professional handlers and household pig owners (14.3%-28.6% vs. 3.7%) suggests that while proximity to pigs is a prerequisite for zoonotic transmission, it is the intensity and type of contact that are the ultimate determinants of infection. This nuanced understanding is critical for public health messaging, as it shifts the focus from general pig ownership to the specific hazardous tasks involved in the pork production chain. Our findings on occupational risk align with trends from other Indonesian regions like Bali and West Kalimantan, where pig handlers also show elevated seroprevalence (9, 10). The public health implications of these findings are direct and actionable. Prevention efforts should target high-risk occupational groups. Simple, cost-effective interventions, such as promoting glove and protective footwear use, implementing stringent hand hygiene protocols, and educating workers about zoonotic HEV risks, could significantly reduce transmission (20). Feasible local interventions include PPE awareness campaigns and improved abattoir hygiene standards.

This study has several limitations. The cross-sectional design establishes association but cannot confirm causality. The small sample number, particularly in the butchers' group, was modest, limiting the statistical power of our prevalence estimates. Future longitudinal studies with larger cohorts are needed to confirm these risk factors and calculate precise odds ratios. Additionally, our testing was limited to IgG, indicating lifetime exposure, and lacked molecular confirmation via HEV RNA PCR or parallel testing of local swine. Future work should address these aspects to confirm active infection and cross-species linkage.

Conclusion

Occupational exposure is a major risk factor for HEV infection in Palangka Raya, with butchers and pig farm workers being at the highest risk. This confirms that close, unprotected contact with pigs and their products is a critical pathway for zoonotic transmission in this region. Public health strategies should be prioritized for these high-risk groups, focusing on education about zoonotic risks, promoting consistent hand hygiene, and encouraging the use of personal protective equipment.

Acknowledgments

The authors would like to thank the University of Palangka Raya and the study participants in Palangka Raya for their time and contribution. We also extend our gratitude to the field and laboratory staff at the Institute of Tropical Diseases, Universitas Airlangga, for their technical support.

Conflict of Interest

The authors declare that they have no competing interests.

Ethical Approval

The study protocol was reviewed and approved by the Ethics Committee of the Faculty of Medicine, Universitas Airlangga (Approval No: 012/EC/KEPK/FKUA/2012).

Artificial Intelligence Statement

The author(s) declare that they have not used any Artificial Intelligence (AI) tools in the creation of this article.

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