

ORIGINAL RESEARCH ARTICLE

Evaluation of chicken egg yolk immunoglobulin Y-based ELISA for serodiagnosis of hepatitis E virus in swine

Arnaud Fondjo Kouam^{1,2*}, **Philippe Herman Nfombouot Njitoyap²**,
Brice Fredy Nemg Simo³, **Cromwel Tepap Zemnou²**,
Kerinyuy Juliene Kongnyuy¹, **Armel Jackson Seukep¹**,
Elisabeth Menkem Zeuko'o¹, **Frédéric Nico Njyou²**,
and **Paul Fewou Moundipa²**

¹Department of Biomedical Sciences, Faculty of Health Sciences, University of Buea, Buea, Southwest Region, Cameroon

²Department of Biochemistry, Faculty of Science, University of Yaoundé 1, Yaoundé, Centre Region, Cameroon

³The Centre for Research in Infectious Diseases, Yaoundé, Centre Region, Cameroon

Abstract

Hepatitis E virus (HEV) is a major zoonotic pathogen, and pigs are an important reservoir. Serology for anti-HEV antibodies poorly reflects active infection, while reverse transcription polymerase chain reaction (RT-PCR) is sensitive but not always feasible for large-scale surveillance. This study aimed to develop an immunoglobulin Y (IgY)-based sandwich enzyme-linked immunosorbent assay (ELISA) for direct detection of HEV antigen (HEV-Ag). An antigenic epitope from the HEV open reading frame 2 capsid protein was identified *in silico*, synthesized, and used to immunize laying hens; IgY was purified from egg yolk and characterized by sodium dodecyl sulfate–polyacrylamide gel electrophoresis. An in-house sandwich ELISA was subsequently developed using the anti-HEV IgY antibodies. Its diagnostic performance was evaluated against RT-PCR using 350 field-collected pig serum samples and compared with commercial anti-HEV IgG and IgM ELISA kits. Immunization yielded high-purity IgY antibodies, with antigen-specific antibody kinetics showing a significant increase in specific immunoreactivity from week 4 onward and peak sensitivity at weeks 6–8 post-immunization. The anti-HEV IgY showed high specificity, with no cross-reactivity observed against a heterologous antigen (HBsAg). The optimized sandwich ELISA detected HEV-Ag with a sensitivity of 95.4% and a specificity of 100% relative to RT-PCR. The prevalence of HEV-Ag was 17.7% (62/350), which was not significantly different from RT-PCR (18.6%, 65/350; $p=0.250$). In contrast, commercial IgG and IgM ELISAs showed high seroprevalence rates (34.9% and 28.3%, respectively) but exhibited significant discrepancies with RT-PCR ($p<0.0001$), indicating limited utility for identifying active infection. Overall, the developed IgY-based sandwich ELISA demonstrated high diagnostic performance for detecting active HEV infection. This assay represents a viable alternative to RT-PCR for large-scale surveillance, particularly in resource-constrained settings, and may contribute to improved control of zoonotic HEV transmission.

*Corresponding author:

Arnaud Fondjo Kouam
(kouam.fondjo@ubuea.cm)

Citation: Kouam AF, Njitoyap PHN, Simo BFN, *et al.* Evaluation of chicken egg yolk immunoglobulin Y-based ELISA for serodiagnosis of hepatitis E virus in swine. *Microbes & Immunity*. 2026;3(3):025470126. doi: 10.36922/MI025470126

Received: November 21, 2025

Revised: December 28, 2025

Accepted: January 26, 2026

Published online: February 19, 2026

Copyright: © 2026 Author(s). This is an Open-Access article distributed under the terms of the Creative Commons Attribution License, permitting distribution, and reproduction in any medium, provided the original work is properly cited.

Publisher's Note: AccScience Publishing remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Keywords: Hepatitis E virus; IgY antibodies; Sandwich ELISA; Antigen detection; Pig serum; Diagnostic performance

1. Introduction

Hepatitis E virus (HEV) is a leading cause of acute viral hepatitis globally and represents a substantial public health burden, especially in regions with limited resources. Its zoonotic potential further elevates its significance as a pathogen of concern.^{1,2} HEV belongs to the family *Hepeviridae* and is categorized into eight genotypes. Genotypes 1 and 2 are predominantly restricted to human infections, whereas genotypes 3 and 4 are zoonotic, with domestic pigs serving as a major reservoir and an important source of human transmission.^{3,4} Human infections commonly occur through the consumption of undercooked pork, exposure to contaminated water, or direct contact with infected animals.^{3,5-7} The high seroprevalence of HEV in swine populations, ranging from 30% to 90% across various regions globally, underscores the considerable risk this virus poses to food safety.⁷⁻¹⁰

Although HEV infection in pigs is largely asymptomatic, the virus actively replicates in the liver and is shed into the environment via bile and feces, facilitating efficient viral dissemination.¹¹ In humans, most infections are also asymptomatic; however, nearly 20% of cases may progress to acute or chronic liver disease, with symptoms ranging from self-limiting jaundice to fulminant hepatic failure.^{12,13} Certain populations, including immune compromised individuals and pregnant women, are at increased risk of severe or chronic hepatitis outcomes.^{14,15} The global impact of HEV infection is substantial, with an estimated 20 million infections annually, resulting in nearly 60,000 deaths.² This profound burden on both public and animal health highlights the critical need for robust surveillance and diagnostic tools to monitor viral circulation in pig reservoirs and to mitigate zoonotic transmission.

The HEV genome is approximately 7.2 kb in length and contains three open reading frames (ORFs). ORF1 and ORF3 encode non-structural proteins and a phosphoprotein involved in viral replication and pathogenesis, respectively. In contrast, ORF2 encodes the viral capsid protein, which is the primary target for host antibody responses.¹⁶⁻¹⁸ The immunodominant conformational epitopes on this capsid antigen (HEV-Ag) elicit both neutralizing and non-neutralizing antibodies.^{19,20} Serological assays detecting anti-HEV IgG and IgM antibodies—most commonly enzyme-linked immunosorbent assay (ELISA)—are widely used in seroprevalence studies to determine population-level exposure.^{7,9,10} While antibody detection can aid in diagnosis when used appropriately, its utility for identifying active infection is limited by several factors, including the critical timing of sample collection, antibody persistence after viral clearance, potential cross-reactivity, and variable host immune responses. These limitations can

lead to substantial interpretation challenges, complicating the distinction between true-positive, true-negative, false-positive, and false-negative results.²¹ Accordingly, the development of diagnostic methods that enable direct detection of HEV is essential.

Although reverse transcription polymerase chain reaction (RT-PCR) of viral RNA remains the gold standard for confirming active HEV replication, its reliance on highly trained personnel and the need for costly imported reagents and equipment limit its practicality for routine surveillance in resource-constrained settings.²² An alternative approach is the detection of viral antigens. In this context, chicken egg yolk immunoglobulin Y (IgY) offers a promising platform for diagnostic assay development. Compared with mammalian IgG, IgY exhibits high antigen-binding affinity, can be produced non-invasively and cost-effectively in large quantities, and does not interfere with mammalian complement systems.^{23,24} Based on these advantages, we hypothesize that IgY antibodies raised against a novel, rationally designed antigenic epitope from the HEV ORF2-encoded capsid protein could effectively detect HEV-Ag in serum. The objective of this study was to develop a sensitive, affordable, and reliable novel IgY-based sandwich ELISA for the efficient detection of HEV-Ag, with the overall goal of improving the diagnosis and surveillance of active infection. The scientific novelty of this work lies in the integrated application of *in silico* epitope prediction and design, followed by the generation and diagnostic application of specific chicken IgY, thereby creating a novel diagnostic reagent and assay format for the direct detection of active HEV infection.

2. Materials and methods

2.1. Reagents

Commercial HEV IgG and IgM ELISA 3.0 (RUO) kits were obtained from MP Biomedicals (Singapore). Horseradish peroxidase (HRP)-conjugated goat anti-porcine IgG (Catalog No. AP166P), HRP-conjugated rabbit anti-chicken IgY (Catalog No. 12-341), Freund's complete adjuvant (CFA) (Catalog No. F5881), and Freund's incomplete adjuvant (IFA) (Catalog No. F5506) were purchased from Sigma-Aldrich (United States of America [USA]). An HRP-conjugated goat anti-pig IgM secondary antibody (Catalog No. MBS224946) was acquired from MyBiosource.com (USA). The QIAamp Viral RNA Mini Kit was sourced from Qiagen (Germany). The SuperScript™ III One-Step RT-PCR System with Platinum™ Taq DNA Polymerase, tetramethylbenzidine (TMB; Catalog No. 002023), and PageRuler™ Prestained Protein Ladder (10–180 kDa; Catalog No. 26617) were procured from Thermo Fisher Scientific (USA). Primer

sequences targeting the HEV ORF2 region (Forward_HEV-5260: 5'-RGTRGTTTCTGGGGTGAC-3'; Reverse_HEV-5330: 5'-AKGGRTTGGTTGGRTGAA-3'; Probe: (HEV5283: 5'-FAM-TGAYTCYCARCCCTTCGC-TAMRA-3') used for HEV RNA detection were adopted from²⁵ and supplied by TSINGKE Biological Technology (China). The peptide representing a dominant HEV capsid antigen epitope identified in this study, along with two dominant antigenic epitopes located at the major hydrophilic region of hepatitis B virus surface antigen (HBsAg; P1: CTTPAQGNSMYPSCCCSKPSDGNC and P2: CMICVQGTSMFPSCCCTKPTDGNC)²⁶ for specificity testing, was synthesized by GenScript (USA). All other reagents were of analytical grade and obtained from commercial suppliers.

2.2. Generation of antigenic peptide epitopes from the HEV viral capsid protein

Antigenic peptide epitopes were generated from the HEV viral capsid protein using an established computational design method.²⁷ Briefly, protein sequences from all HEV genotypes were retrieved in FASTA format from the National Center for Biotechnology Information database. To ensure a non-redundant dataset, sequences were subjected to a Basic Local Alignment Search Tool analysis to identify and remove duplicates and highly similar entries.

The refined sequences were aligned using the CLUSTAL Omega algorithm within the BioEdit software to identify conserved regions and generate consensus sequences. The antigenic potential of these consensus sequences was predicted using the VaxiJen v.2.0 server with a threshold of 0.5.²⁸ Subsequently, subcellular localization was predicted using the Virus-mPLOC server, a multi-label predictor for viral proteins.²⁷ Then, to assess potential safety concerns, the allergenicity of the candidate peptides was evaluated using the AllerTOP v.2.0 server, a machine learning-based tool that compares peptide sequence features with known allergens.²⁸⁻³⁰ Finally, the antigenic peptide sequence exhibiting the highest predicted antigenicity and lowest allergenicity was selected for chemical synthesis and subsequent immunization of laying hens.

2.3. Immunization of laying hens

Three 6-month-old Lohmann Brown Classic laying hens were obtained from the AGROCAM Poultry Complex (Foumbot, Cameroon). The hens were housed in cages (150 × 100 × 80 cm) within the animal facility of the Pharmacology Laboratory, Faculty of Health Sciences, University of Buea. They were provided with layer feed (250 ± 10 g/hen/day) and tap water *ad libitum*. All procedures were approved by the Institutional Animal Care and

Use Committee of the University of Buea (UB-IACUC N°59/2023). The immunization protocol consisted of three weekly intramuscular injections of 0.5 mL peptide antigen (1 mg/mL) per hen. The primary immunization was administered with the antigen emulsified in CFA, followed by two booster injections using IFA. Pre-immune eggs (7/hen) were collected 1 week before the first immunization. Egg collection continued for 8 weeks post-initial immunization for subsequent IgY extraction.

2.4. Extraction of IgY from eggs

A soluble fraction enriched for antigen-specific IgY was isolated from egg yolks via a modified salt precipitation protocol.³¹ For each weekly egg collection, yolks were separated, rinsed with distilled water, and pooled in a measuring cylinder. The pooled yolks were diluted 1:1 (v/v) with 0.1 M phosphate-buffered saline (PBS) containing 0.1% sodium azide (Sigma-Aldrich, USA). This mixture was further diluted with eight volumes of distilled water, and the pH was adjusted to 5.0 using 0.5 N hydrochloric acid (Sigma-Aldrich, USA) before being frozen at -20°C. After thawing, the sample was centrifuged (Centrifuge 5804 R, Eppendorf, Germany) (4,000 × g, 30 min), and the supernatant was filtered (0.22 µm). Sodium chloride (Sigma-Aldrich, USA) was then added to the filtrate to a final concentration of 8.8% (w/v), and the pH was lowered to 4.0. Following a 2-h incubation at room temperature, the solution was centrifuged again (3,700 × g, 30 min, 4°C). The resulting pellet was resuspended in PBS to 10% of the original filtrate volume, yielding the soluble IgY fraction, which was aliquoted and stored at -80°C until used. IgY concentration and purity were assessed using the Lowry method and sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) analysis, respectively.

2.5. Determination of protein concentration in the specific IgY fraction

The protein concentration of the extracted antigen-specific IgY fraction was quantified using the Lowry method.³² This assay involves two sequential chemical reactions: first, the Biuret reaction, in which copper ions form a complex with peptide bonds under alkaline conditions and are reduced to cuprous ions; second, the Folin-Ciocalteu reaction, in which these reduced ions catalyze the reduction of a phosphomolybdic-phosphotungstic acid complex, resulting in a blue chromophore. The absorbance of this solution at 750 nm is directly proportional to the protein concentration.

For the assay, 300 µL of 10-fold diluted IgY fraction (assay) or PBS (blank) was combined with 2.5 mL of Biuret's reagent and incubated for 10 min at room temperature. Subsequently, 0.5 mL of Folin-Ciocalteu reagent (Sigma-

Aldrich, USA) was added, and the mixture was incubated in the dark for 30 min. Absorbance was measured at 750 nm against the blank, and the protein concentration was interpolated from a standard curve prepared with bovine serum albumin (Sigma-Aldrich, USA).

2.6. Analysis of the antigen-specific IgY fraction by SDS-PAGE

The identity and purity of the isolated antigen-specific IgY were confirmed by SDS-PAGE, which separates proteins based on their molecular weight under denaturing conditions. Discontinuous gels were prepared by polymerizing a 10% resolving gel, overlaid with ethanol (Sigma-Aldrich, USA) to achieve a flat surface. After polymerization, the ethanol was discarded and replaced with a 5% stacking gel into which a comb was inserted to form wells. The IgY samples were prepared by dilution in 5× Laemmli buffer (with or without dithiothreitol) (Sigma-Aldrich, USA), heated at 100°C for 10 min, and briefly centrifuged. Aliquots of 10 µL (containing approximately 50 µg of protein) were loaded onto the gel alongside a molecular weight marker. Electrophoresis was carried out at a constant voltage of 140 V for 60 min. Following separation, the proteins were visualized by staining with Coomassie Blue dye (Sigma-Aldrich, USA).

2.7. Evaluation of immunoreactivity in specific IgY fractions

The antigen-binding capacity of the extracted IgY fractions was assessed via an indirect ELISA, targeting a representative HEV-Ag peptide epitope. Briefly, 96-well microplates were coated with 100 µL of a solution containing 2 µg of the peptide antigen in a 50 mM carbonate-bicarbonate coating buffer (pH 9.6) (Sigma-Aldrich, USA) and incubated overnight at 4°C. To prevent non-specific binding, the plates were then blocked with 100 µL of 5% (w/v) skim milk (Nestle, Cameroon) prepared in PBS (pH 7.4) for 1 h at room temperature.

Following a wash step, 100 µL of the primary IgY sample was added to each well and incubated for 2 h. Plates were subsequently washed twice with PBS containing 0.05% Tween-20 (PBST). HRP-conjugated goat anti-chicken IgG secondary antibody was then added (100 µL/well) and incubated for 1 h. After two additional PBST washes, the enzymatic reaction was initiated by adding 100 µL of TMB substrate and incubating for 15 min in the dark. The reaction was terminated with 50 µL of 2 M sulfuric acid (H₂SO₄) (Sigma-Aldrich, USA), and optical density (OD) was immediately read at a wavelength of 450 nm using a microplate reader (Multiskan FC spectrophotometer, Thermo Scientific, USA).

2.7.1. Determination of the appearance time of antigen-specific IgY in the extracted IgY fractions

To determine the kinetics of the immune response, all IgY fractions, from pre-immune eggs through to those collected up to 8 weeks post-initial immunization, were analyzed at a fixed concentration of 10 µg per well using the ELISA protocol described above. A sample was confirmed as positive for antigen-specific IgY if its OD_{450 nm} value exceeded 2.5-fold the background OD measured using the pre-immune IgY control.

2.7.2. Assessment of sensitivity and specificity

The sensitivity of the antigen-specific IgY fractions was evaluated using a concentration-dependent assay. Serial two-fold dilutions of the specific IgY (starting from 10 µg per well in a 100 µL volume) were tested. The endpoint titer was defined as the lowest concentration at which the OD₄₅₀ value remained greater than twice that of the highest concentration of pre-immune IgY.

To evaluate specificity and potential cross-reactivity, a parallel assay was conducted. A separate plate was coated with a 2 µg/well of a peptide antigen epitope from HBsAg. The immunoreactivity of the HEV-specific IgY fractions was tested on both the HEV-Ag and HBsAg-coated plates. The resulting OD values for each concentration were directly compared to ascertain binding specificity.

2.8. Collection of blood samples and serum preparation

Between September 2023 and April 2024, a total of 350 blood samples were randomly obtained from slaughter-age pigs at the exsanguination point of the abattoir in Bonaberi-Douala, the economic capital of the Republic of Cameroon. The inclusion criteria were pigs designated for routine slaughter, with no overt signs of severe systemic illness at ante-mortem inspection. Animals showing obvious signs of acute febrile illness, severe weakness, or those that were not part of the standard commercial slaughter process were excluded from the study. The sampling aimed to represent the general population of market-weight pigs, which are the primary reservoir for zoonotic HEV transmission. Blood was drawn directly into pre-labeled, anticoagulant-free tubes (numbered 001 to 350) and immediately placed in a cool box maintained at 4°C for transport. On arrival at the laboratory, the samples were left to clot for 30 min at ambient temperature. Subsequently, the tubes were centrifuged (3000 × g for 10 min at 4°C) to separate the cellular components. The resulting supernatant (serum) was aliquoted into cryovials and stored at –80°C until further analysis.

2.9. Viral RNA extraction and molecular detection of HEV via real-time RT-PCR

To confirm the presence of actively circulating HEV within the pig population, all serum samples were first screened for HEV RNA targeting the ORF2 region using specific primers. Viral RNA was isolated from 140 μ L of each serum sample using the QIAamp Viral RNA Mini Kit (Qiagen, Germany), by adhering to the manufacturer's protocol. Subsequent detection of HEV RNA was carried out by real-time RT-PCR on an Applied Biosystems 7500 platform (Thermo Fisher Scientific, USA). The assays were performed using the SuperScript[™] III One-Step RT-PCR System with Platinum[™] Taq DNA Polymerase, as previously established.²⁵ Each 50 μ L reaction contained 10 μ L of the extracted RNA template, 0.4 μ M of each primer, 2.3 mM magnesium sulfate, and 200 μ M deoxynucleotide triphosphates. The thermocycling conditions were initiated with a reverse transcription step at 50°C for 30 min. This was followed by an initial denaturation at 94°C for 2 min, and 45 cycles of amplification (denaturation at 94°C for 15 s, annealing at 51°C for 1 min, and extension at 72°C for 1 min). A final extension was performed at 72°C for 5 min. Fluorescence data were acquired at the end of each annealing/extension step. A sample was considered positive for HEV RNA if a characteristic sigmoidal amplification curve emerged from the baseline with a cycle threshold before the 40th cycle.

2.10. Serological detection of anti-HEV IgM and IgG using commercial ELISA kits

The presence of anti-HEV IgM and IgG antibodies in the serum samples was analyzed using commercial ELISA kits. The manufacturer's protocol was followed, with the modification that the provided enzyme-conjugated anti-human secondary antibodies were substituted with HRP-conjugated goat anti-pig IgM and goat anti-porcine IgG antibodies to ensure species-specific detection. In brief, serum samples were diluted at 1:50 (v/v) and added to the microtiter plate wells pre-coated with recombinant HEV antigens. The plate was incubated at 37°C for 60 min and subsequently washed to remove unbound proteins. For the detection of specific Ig isotypes, the respective HRP-conjugated secondary antibody (anti-pig IgM or anti-porcine IgG) was added to each well and incubated at 37°C for 60 min. Following another wash cycle, 100 μ L of 0.1 M TMB substrate solution was added to each well, initiating an enzymatic reaction that produced a blue color. After a 30-min incubation at 37°C in the dark, the reaction was stopped by adding 50 μ L of 2 M H₂SO₄, resulting in a color change from blue to yellow. The absorbance of each well was measured at 450 nm. Each assay included a blank, and the negative and positive controls supplied with the kit.

The cut-off value (COV) for each plate was calculated as the mean absorbance of three negative control replicates plus a constant (0.250 for IgM and 0.350 for IgG). Results were expressed as a ratio of sample absorbance (As) to COV. Samples were interpreted as positive for anti-HEV antibodies if the As/COV ratio exceeded 1.200 for IgM or 1.100 for IgG.

2.11. Diagnostic evaluation of antigen-specific IgY in detecting HEV-antigen in serum through a sandwich-ELISA

To evaluate the diagnostic potential of the most immunoreactive antigen-specific IgY, an in-house sandwich-ELISA was developed. For initial evaluation, 10 RT-PCR-positive and 10 RT-PCR-negative serum samples were randomly selected. The assay was optimized by coating a 96-well immuno-plate with varying concentrations of the specific IgY (1.25, 2.5, and 5 μ g per 100 μ L). Non-specific binding sites were subsequently blocked with 5% dehydrated skim milk. The protocol incorporated control wells, including a blank reagent and IgY derived from pre-immune eggs. Each well was incubated with 100 μ L of a 50-fold diluted serum sample for 2 h. After two washes with PBST, 5 μ g/mL of the specific IgY was introduced and allowed to incubate for another 2 h. Following another wash cycle, HRP-conjugated goat anti-chicken IgG secondary antibody was added for 2 h. After a final wash cycle, the enzymatic reaction was initiated by adding 100 μ L of 0.1 M TMB substrate and incubating for 30 min in the dark. The reaction was terminated by the addition of 50 μ L of 2 M H₂SO₄, and the OD was read at 450 nm. The COV for HEV-Ag positivity was defined as twice the highest OD value obtained from pre-immune IgY controls. Based on the outcomes of this initial evaluation, the optimal IgY coating concentration was selected for subsequent analysis of all serum samples.

2.12. Statistical analysis

To minimize analytical bias, all laboratory analyses were performed in a blinded manner. Personnel performing RT-PCR, commercial ELISA, and the in-house IgY-based ELISA were unaware of the results from the other tests until all laboratory data had been finalized and the blinding codes were released for statistical analysis. Results of all laboratory tests for each blood sample were compiled and recorded in Microsoft Excel 2016 (Microsoft Corporation, USA) (Supplementary File S1) before export into the appropriate software for data analysis. Quantitative data are expressed as the mean $\pm$ standard deviation of three independent experiments, each performed in triplicate. Qualitative data are presented as frequency and percentage (%). Comparisons of means between

two groups were performed using an unpaired Student's *t*-test. Comparisons among more than two groups were analyzed using one-way analysis of variance (ANOVA), followed by Bonferroni's *post hoc* test. Comparisons of frequency distributions (proportions) between two categorical variables were assessed by cross-tabulation followed by Chi-square or McNemar's test. A $p < 0.05$ was considered statistically significant. All statistical analyses were performed using GraphPad Prism (version 8.0.2) (GraphPad Software, USA) and IBM SPSS Statistics (version 25.0) (IBM Corp., USA).

3. Results

3.1. Prediction of antigenic peptide epitopes from HEV capsid protein sequences

Using the CLUSTAL Omega algorithm implemented in BioEdit, we generated consensus sequences representing potential antigenic epitopes of the HEV capsid protein. Based on the predefined selection criteria, three peptides were identified and designated as P1 (111 aa), P2 (660 aa), and P3 (111 aa) (Table 1). Antigenicity analysis using the VaxiJen server yielded scores of 0.7457 for P1, 0.6291 for P2, and 0.5138 for P3, all exceeding the recommended threshold of 0.5, indicating good antigenic potential. Subcellular localization predictions performed using the

Virus-mPloc server indicated that P1 localizes to the host cell membrane, P2 to the host cytoplasm, and P3 to the viral capsid. Furthermore, allergenicity assessment using the AllerTOP server predicted that none of the peptides were allergenic (Table S1 and Figures S1-S4). Based on its highest predicted antigenicity score, peptide P1 was selected for synthesis and subsequently used as an antigen for immunizing laying hens.

3.2. Protein content and purity of egg yolk IgY fractions following immunization

The total protein concentration of the extracted IgY fraction was quantified using the samples collected from pre-immune eggs and eggs obtained weekly for 8 weeks post-immunization. Immunization with the HEV-Ag antigenic peptide did not significantly ($p > 0.05$) alter the total protein content of the IgY fraction at any post-immunization time-point compared with pre-immune eggs (Figure 1A). Across the study period, IgY concentrations remained consistent, ranging from 31.49 ± 2.7 mg/mL at week 1 to 34.64 ± 2.2 mg/mL at week 7.

The successful extraction and purity of IgY were confirmed by SDS-PAGE (Figure 1B). Under non-reducing conditions, a single prominent band was observed at approximately 180 kDa, corresponding to the molecular weight of intact IgY. Under reducing conditions,

Table 1. Antigenic peptide epitopes identified from HEV capsid protein sequences

S/N	Antigenic peptide epitopes sequences	Number of amino acids	Subcellular localization	Antigenicity score*	Allergenicity
1	MFCIHGSPVNSYNTNPTGTALGLLDFALELEFRNLTPGNTNTRVSR TSTARHRLRRGADGTAELTTAATRFMKDLHFTGT NGVGEVGRGIALTLFNLADTLGGLPTEL	111	Host cell membrane	0.7457	Non-allergenic
2	MRPRAVLLFFVLLPMLPAPPAGQPSGRRRRGRS GGAGGGFWGDRVDSQPALPYIHPNPEAADVVSQSGAGARPRQPAR PLGSSWRDQSQRPAAAPRRRSTPAGAAPLTAVSPAP DTAPVPDVDSRGAILRRQYNLSTSPLTSSVASGTNLVLYAAPLNPLPL QDGTNTHIMATEASNYAQYRVVRATIRYRPLVP NAVGGYAISISFWPQT'TTPTSDMNSITSTDVRLVQPGIASLVIPS ERLHYRNQGWRSVETSGVAEEATSGLVMLCIH GSPVNSYNTNPTGTALGLLDFALELEFRNLTPGNTNTRVSRYTSTAR HRLRRGADGTAELTTAATRFMKDLHFTGTN GVGEVGRGIALTLFNLADTLGGLPTELISSAGGQLF YSRPVVSANGEPTV KLYTSVENAQQDKGIAIPHDIDLGDSRVVIQDYDNQHEQD RPTSPAPSRPFSVLRANDVLWLSLTAAYDQTTYGSSTNPM YVSDTVTFVNVATGAQAVARSLDWSKVTL DGRPLTIQQYSKTFYVLP LRGKLSFWEAGTTKAGYPYNYNTTASDQILIENAA GHRVAISTYTTSLGAGPVSVAAGVGLAPHSALAVLEDTIDYPARAHT FDDFCPCRNLGLQGCAFSQSTIAELQRLKMKVKGKTRES	660	Host cytoplasm	0.6291	Non-allergenic
3	SPCALGLFCCCSCFCLCCPRHRPVSRLLAAVVGAAAVPAVSVGTGL ILSPSPSIFIQPTSPPMSPRNPGLALDSPAAPLGVTSPSJ APPLPHVVDLPQLGLRR	111	Viral capsid	0.5138	Non-allergenic

*Antigenicity (cut-off=0.5). HEV: Hepatitis E virus.

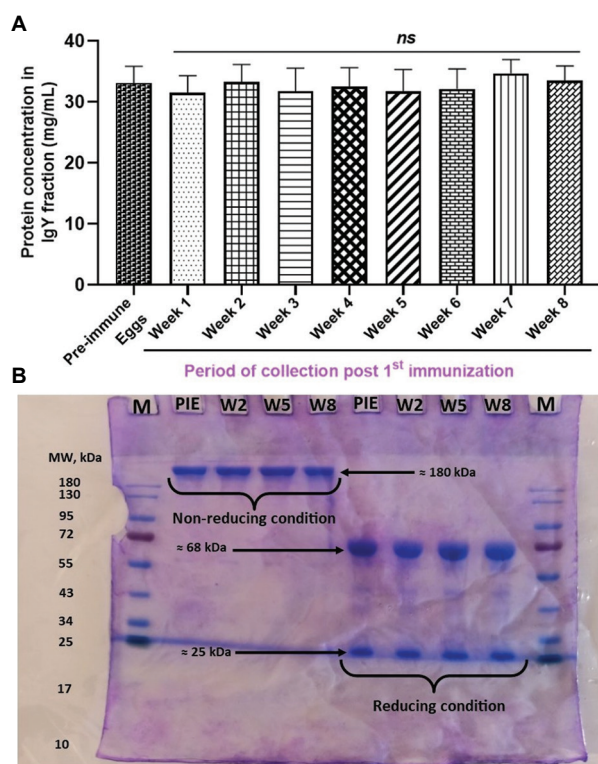


Figure 1. Protein content and sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) analysis of the egg yolk IgY fraction. (A) Total protein concentration in the extracted IgY fraction. Data are expressed as mean $\pm$ SD. Statistical analysis was performed using one-way ANOVA followed by Bonferroni's *post hoc* test; ns indicates a non-significant difference ($p > 0.05$) compared to the pre-immune eggs group. (B) Representative SDS–PAGE gel under non-reducing and reducing conditions.

Notes: M: Molecular weight marker; PIE: IgY fraction from pre-immune eggs; W2, W5, W8: IgY fraction from eggs collected at weeks 2, 5, and 8 post-immunization, respectively.

the IgY fraction separated into two predominant bands at approximately 68 kDa and 25 kDa, consistent with the expected molecular weight of the heavy and light chains, respectively. The overall purity of the extracted IgY was estimated to exceed 95%, demonstrating the effectiveness of the purification protocol.

3.3. Immunoreactivity of the extracted antigen-specific IgY fractions

3.3.1. Kinetics of antigen-specific IgY appearance in egg yolks

An indirect ELISA was developed to assess the immunoreactivity of the extracted IgY fractions against the HEV antigenic peptide epitope. To determine the kinetics of the specific antibody response, all IgY fractions—from pre-immune eggs through to those collected 8 weeks post-initial immunization—were analyzed at a fixed concentration of 10 μ g/well against plates coated with

2 μ g/well of antigen. The COV for positivity (OD_{450} nm) was established at 0.648 ± 0.05 , defined as twice the highest OD value obtained using pre-immune IgY.

As shown in Figure 2A, IgY from immunized hens exhibited a gradual increase in OD values over time. During weeks 1–3 post-immunization, OD values remained marginally above the pre-immune baseline. A significant increase ($p < 0.05$) was first observed at week 4 and persisted through week 8. All IgY fractions collected from weeks 4 to 8 significantly exceeded the established COV and continued to increase, indicating that the production and accumulation of HEV-specific IgY were first detectable in egg yolks at week 4, following the third immunization. Based on this kinetic profile, IgY fractions from weeks 4–8 were selected for subsequent sensitivity analysis.

3.3.2. Concentration-dependent reactivity of antigen-specific IgY

The sensitivity of IgY fractions from weeks 4 to 8 was evaluated using a concentration-response assay. Serial two-fold dilutions of each fraction (10 to 0.019 μ g/100 μ L) were tested against plates coated with 2 μ g/well of HEV antigen. As illustrated in Figure 2B, all fractions demonstrated a clear concentration-dependent binding profile. IgY fractions from weeks 4 and 5 exhibited the lowest sensitivity, with endpoint titers of 5 μ g/100 μ L and 2.5 μ g/100 μ L, respectively. In contrast, IgY from weeks 6–8 demonstrated markedly higher sensitivity, with endpoint titers of 0.625 μ g/100 μ L for week 6 and 0.312 μ g/100 μ L for both weeks 7 and 8. Given their superior sensitivity, IgY fractions from weeks 6–8 were selected for specificity assessment.

3.3.3. Specificity of the HEV antigen-specific IgY fractions

The specificity of the selected HEV-specific IgY fractions (weeks 6–8) was assessed by comparing their immunoreactivity toward the target HEV-Ag and a heterologous control antigen (HBsAg). IgY fractions were tested at concentrations of 0.625, 1.25, 2.5, and 5 μ g/100 μ L on plates coated with either HEV-Ag or HBsAg (2 μ g/well).

As illustrated in Figure 2C, the reactivity of the HEV-specific IgY with HBsAg remained consistent across all concentrations tested, indicating minimal cross-reactivity. In contrast, binding to HEV-Ag (OD values) increased in a concentration-dependent manner. At each corresponding concentration, OD values obtained for HEV-Ag were significantly higher ($p < 0.05$) than those observed for HBsAg. These findings confirm the high binding specificity of the extracted IgY fractions for the target HEV epitope.

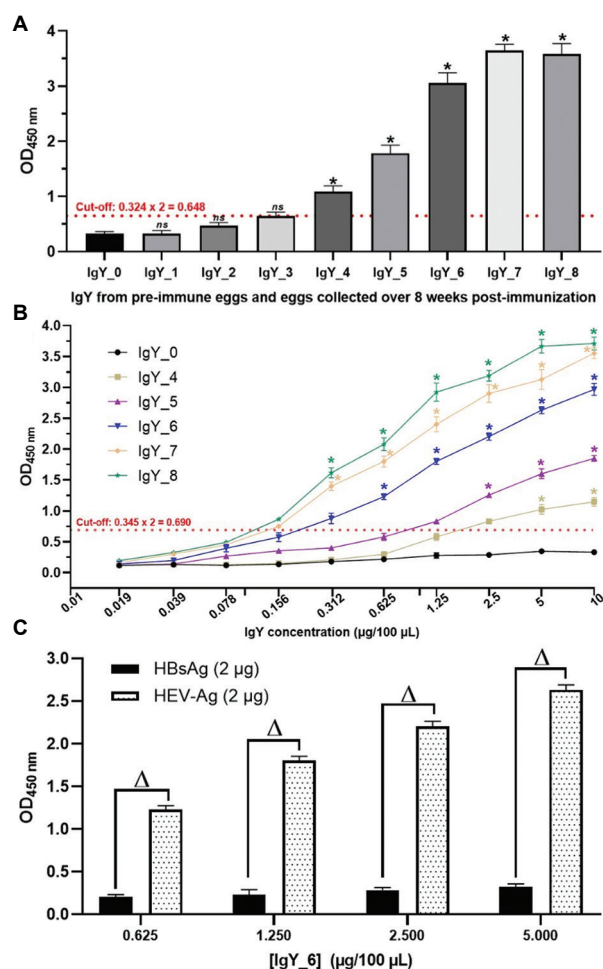


Figure 2. Indirect enzyme-linked immunosorbent assay (ELISA) analysis of antigen-specific immunoreactivity of IgY fractions. IgY was extracted from pre-immune eggs and eggs collected over 8 weeks post-immunization. Their reactivity towards hepatitis E virus (HEV) and hepatitis B virus (HBV) (for specificity test) antigenic peptide epitopes was assessed by indirect ELISA (OD 450 nm). (A) Kinetics of specific anti-HEV IgY appearance in egg yolks. (B) Sensitivity of the antigen-specific IgY response, shown by concentration-dependent reactivity. (C) Specificity of the IgY fractions, tested against HBsAg and HEV-Ag antigenic peptide epitopes.

Notes: Data are presented as mean $\pm$ SD ($n=3$ independent experiments, each in triplicate). Statistical annotations: ns denotes $p>0.05$ vs. cut-off value (one-way analysis of variance [ANOVA] with Bonferroni's *post hoc* test); *denotes $p<0.05$ vs. cut-off value (one-way ANOVA with Bonferroni's *post hoc* test); Δ denotes $p<0.05$ between indicated groups (unpaired Student's *t*-test). IgY_0: IgY fraction from pre-immune eggs; IgY_1,2,3,...,8: IgY fraction collected at week 1 to 8 post-immunization.

Accordingly, these fractions were selected for diagnostic evaluation.

3.4. Diagnostic potential of HEV-specific IgY fractions for detecting HEV-antigen in serum

Following confirmation of sensitivity and specificity, an indirect sandwich ELISA was developed to evaluate

the diagnostic potential of the anti-HEV IgY fractions (weeks 6–8) for the detection of HEV-Ag in serum. IgY was coated onto microplates at concentrations of 1.25, 2.5, and 5 $\mu\text{g}/100 \mu\text{L}$ /well. Plates coated with pre-immune IgY served as controls.

As summarized in Table 2, the absorbance values obtained using pre-immune IgY control were consistently low, ranging from 0.177 to 0.270 for both positive ($n=10$) and negative ($n=10$) HEV serum samples. Similarly, the anti-HEV IgY fractions, when incubated with negative serum, yielded a low absorbance range comparable to the pre-immune IgY control, confirming the absence of non-specific binding. In contrast, incubation of the anti-HEV IgY with positive HEV serum resulted in a pronounced, concentration-dependent increase in absorbance. At coating concentrations of 1.25, 2.5, and 5 $\mu\text{g}/100 \mu\text{L}$, OD values ranged from 0.694–1.155, 1.036–1.759, and 1.144–2.116, respectively, across all ten positive samples. The positive cut-off value was defined as 2.5-fold the maximum OD value of the pre-immune control (0.695). At a coating concentration of 1.25 $\mu\text{g}/100 \mu\text{L}$, 7 out of 10 values exceeded the cut-off. Interestingly, at the higher coating concentrations of 2.5 and 5 $\mu\text{g}/100 \mu\text{L}$, all 10 positive samples produced OD values significantly above the cut-off ($p<0.05$), demonstrating successful detection of HEV-Ag. Based on these results, the HEV-specific IgY fraction at a coating concentration of 2.5 $\mu\text{g}/100 \mu\text{L}$ was selected for subsequent screening of all collected serum samples.

3.5. Prevalence and diagnostic performance of serological assays and in-house IgY ELISA antigen test compared to RT-PCR

3.5.1. Seroprevalence of anti-HEV antibodies

Among the 350 blood samples collected, 168 (48.0%; 95% CI: 42.8–53.2) were positive for at least one serological marker of HEV infection (anti-HEV IgG and/or IgM) (Figure 3A). Specifically, anti-HEV IgG was detected in 122/350 samples (34.9%; 95% CI: 29.8–39.9) (Figure 3B), while anti-HEV IgM was detected in 99/350 samples (28.3%; 95% CI: 23.5–33.0) (Figure 3C). A Venn diagram illustrating mono- and co-detections revealed that, among the 168 seropositive samples, anti-HEV IgG mono-detection, anti-HEV IgM mono-detection, and anti-HEV IgG/IgM co-detection accounted for 19.7% (69/350), 13.1% (46/350), and 15.1% (53/350) of the total population, respectively (Figure 3D).

3.5.2. Prevalence of HEV viremia and antigenemia

To identify active HEV infection within the pig population, the presence of HEV RNA and HEV-Ag was assessed. RT-PCR analysis detected HEV RNA in 65 of

Table 2. Detection of HEV-Ag in pig serum specimens by sandwich ELISA using antigen-specific IgY

SerumID	IgY_0 (5 µg/100 µL)		IgY_6 (5 µg/100 µL)	IgY_6		
	Negative HEV serum	Positive HEV serum		1.25 µg/100 µL Positive HEV serum	2.5 µg/100 µL Positive HEV serum	5 µg/100 µL Positive HEV serum
1	0.177	0.178	0.272	1.042*	1.307*	1.729*
2	0.202	0.270	0.181	1.106*	1.473*	1.824*
3	0.179	0.196	0.163	0.787	1.042*	1.298*
4	0.169	0.265	0.210	1.155*	1.759*	2.116*
5	0.186	0.212	0.138	0.694	1.036*	1.144*
6	0.219	0.181	0.169	1.121*	1.614*	1.853*
7	0.178	0.182	0.211	1.120*	1.519*	1.848*
8	0.256	0.182	0.171	1.093*	1.475*	1.804*
9	0.212	0.273	0.203	0.861	1.164*	1.521*
10	0.259	0.176	0.223	1.018*	1.474*	1.685*

Data represent the mean absorbance values of three independent experiments performed in triplicate. The cut-off value for positive detection (dashed line) was set at 0.695 ± 0.1 , defined as 2.5-fold of the highest absorbance value (0.270) obtained with IgY_0, either with negative or positive serum specimens. *Significantly higher than the cut-off value, $P < 0.05$ (one-way ANOVA with Bonferroni's *post-hoc* test), indicating positive detection. IgY_0: IgY fraction from pre-immune eggs. IgY_6: Specific antigen IgY fraction from eggs collected in the 6th week after immunization. Abbreviations: ELISA: Enzyme-linked immunosorbent assay; IgG: Immunoglobulin G; HEV: Hepatitis E virus.

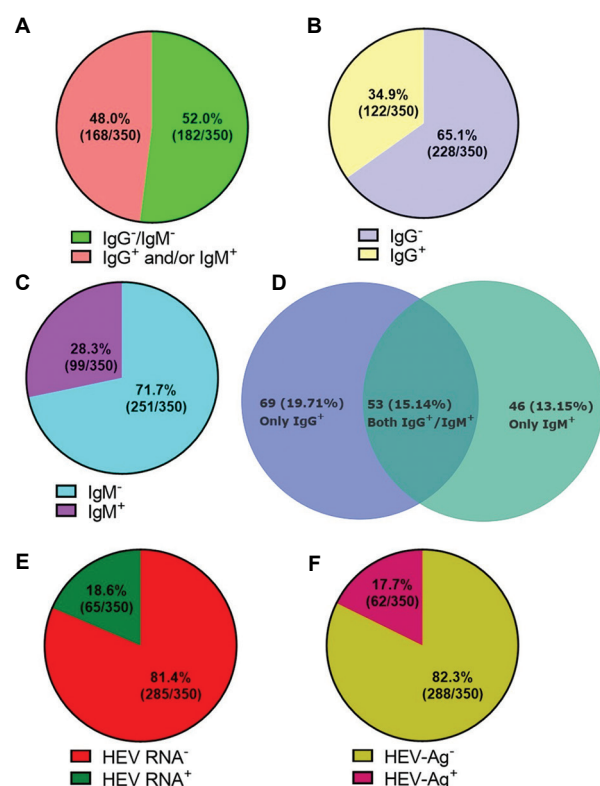


Figure 3. Seroprevalence and virological markers of hepatitis E virus (HEV) infection in pig sera. Serum samples ($n=350$) were analyzed for: (A) Overall anti-HEV seropositivity (IgG and/or IgM). (B) Anti-HEV IgG. (C) Anti-HEV IgM. (D) IgG and IgM mono- and co-detection. (E) HEV RNA by reverse transcription polymerase chain reaction. (F) HEV antigen (HEV-Ag) by the in-house sandwich IgY-Enzyme-linked immunosorbent assay.

the 350 samples (18.6%; 95% CI: 14.5–22.7) (Figure 3E). Similarly, the in-house sandwich IgY-ELISA developed for HEV-Ag detection in serum identified 62 positive samples out of 350 (17.7%; 95% CI: 13.7–21.7) (Figure 3F).

3.5.3. Diagnostic performance of ELISAs compared to RT-PCR

The diagnostic performance of the commercial anti-HEV IgG/IgM ELISA kits, as well as the in-house HEV-Ag ELISA, was evaluated against the RT-PCR gold standard using McNemar's test (Table 3).

The commercial anti-HEV IgG assay showed a significant discrepancy when compared with RT-PCR ($p < 0.0001$). Of the 122 IgG-positive samples, 69 were RT-PCR negative, while 12 of the 228 IgG-negative samples were RT-PCR positive. Similarly, the commercial anti-HEV IgM assay also demonstrated a significant difference relative to RT-PCR ($p < 0.0001$). Among the 99 IgM-positive samples, 42 were RT-PCR negative, while 8 of the 251 IgM-negative samples were RT-PCR positive.

In contrast, the in-house IgY-ELISA HEV-Ag test showed no significant difference compared with RT-PCR ($p=0.250$). All 62 HEV-Ag-positive samples were also RT-PCR positive, and 285 of the 288 HEV-Ag-negative samples were RT-PCR negative. Relative to RT-PCR, the assay showed a sensitivity of 95.4% (62/65) and a specificity of 100% (285/285); PPV and NPV were 100% (62/62) and 98.95% (285/288), respectively. These results indicate that the in-house IgY-ELISA HEV-Ag test is a

Table 3. Cross-tabulation analysis of the diagnostic performance of ELISA tests compared to RT-PCR

Diagnosis methods	Outcomes	RT-PCR HEV RNA			Cross-tabulation McNemar's analysis (<i>p</i> -value)
		Negative, <i>n</i> (%)	Positive, <i>n</i> (%)	Total	
ELISA-IgG	Negative	216 (61.7)	12 (3.4)	228 (65.1)	<0.0001*
	Positive	69 (19.7)	53 (15.1)	122 (34.9)	
	Total	285 (81.4)	65 (18.6)	350 (100.0)	
ELISA-IgM	Negative	243 (69.4)	8 (2.3)	251 (71.7)	<0.0001*
	Positive	42 (12.0)	57 (16.3)	99 (28.3)	
	Total	285 (81.4)	65 (18.6)	350 (100.0)	
ELISA-IgG/IgM	Negative	282 (80.6)	15 (4.3)	297 (84.9)	0.008*
	Positive	3 (0.9)	50 (14.3)	53 (15.1)	
	Total	285 (81.4)	65 (18.6)	350 (100.0)	
In-house Sandwich ELISA-HEV-Ag	Negative	285 (81.4)	3 (0.9)	288 (82.3)	0.250
	Positive	0 (0.0)	62 (17.7)	62 (17.7)	
	Total	285 (81.4)	65 (18.6)	350 (100.0)	

The statistical analysis of proportions was performed using cross-tabulation followed by McNemar's Test. *Denotes significant difference at $P < 0.05$. Abbreviations: RT-PCR: Reverse transcription polymerase chain reaction; ELISA: Enzyme-linked immunosorbent assay; IgG: Immunoglobulin G; HEV: Hepatitis E virus.

promising alternative for the detection of active HEV infection.

4. Discussion

This study reports the successful development and validation of a novel in-house sandwich IgY-ELISA for the detection of HEV-Ag in swine serum. The scientific novelty of this work encompasses three key aspects: (i) The rational *in silico* design of a conserved and highly antigenic HEV capsid epitope; (ii) the use of this designed peptide to generate specific chicken IgY antibodies, representing a non-mammalian and high-yield source of immunoglobulins; and (iii) the deployment of these IgY antibodies in a sandwich ELISA format for the direct, sensitive, and specific detection of viral antigen as a marker of active infection. A key finding of this study is the high diagnostic performance of the IgY-ELISA-based test, which showed no statistically significant difference compared with the RT-PCR gold standard method ($p = 0.250$) and achieved a sensitivity of 100% and a specificity of 98.95%. The practical significance of this assay is substantial, as it provides a viable, high-performance alternative to RT-PCR for large-scale surveillance and point-of-care diagnosis of active HEV infection in swine populations. These results position the IgY-based assay as a reliable, cost-effective, and practical alternative tool for diagnosing active HEV infection, particularly suited for resource-limited settings where the technical complexity and financial burden of molecular methods like RT-PCR impede widespread surveillance. In this context, the IgY-ELISA provides a pragmatic bridge, offering a field-deployable tool with

accuracy approaching that of the gold standard, while retaining the simplicity, speed, and affordability of ELISA-based testing.

A critical foundation underlying the success of this diagnostic assay is the rational *in silico* approach employed for antigen design prior to laying hen immunization. Using a robust computational pipeline, a conserved and highly antigenic epitope (P1) within the HEV ORF2 capsid protein was successfully identified among several consensus sequences generated across various HEV genotypes. Indeed, antigenicity prediction using the VaxiJen server ensured that the selected peptide was capable of eliciting a strong and potentially broad-spectrum immune response. Furthermore, the prediction of non-allergenicity through AllerTOP v2.0 and the confirmation of host cell membrane localization using Virus-mPLOC were crucial steps in selecting a safe and biologically relevant immunogen.^{27,28,30} This rational design strategy likely contributed directly to the high affinity and specificity of the resulting IgY immunoglobulins, as reflected by the minimal cross-reactivity observed and the excellent diagnostic accuracy of the developed assay.

The high overall seroprevalence of anti-HEV antibodies (48.0%) observed in the studied pig population aligns with the well-established role of swine as a major reservoir for zoonotic HEV genotypes.^{3,4} The observed seroprevalence rates of IgG (34.9%) and IgM (28.3%) are consistent with the broad range (30–90%) reported globally, including studies from other sub-Saharan African settings.^{7,9,10,33} This high seroprevalence indicates substantial and continuous

exposure to HEV in Cameroon, involving pigs, humans, and the environment.^{25,34–36} Consequently, these findings underscore a significant risk of zoonotic transmission via the food chain, contaminated water supply, or direct contact with infected animals.^{5,8}

However, our results clearly highlight the limitations of serological antibody-based assays for detecting active infection. The significant discrepancies ($p < 0.0001$) observed between commercial IgG/IgM ELISA results and RT-PCR outcomes confirm that antibody detection alone is an unreliable indicator of active viremia. The high proportion of false-positive IgM results (42 of 99 IgM-positive samples were RT-PCR negative), often considered indicative of recent infection,³⁷ may reflect persistence of IgM beyond the viremic phase or non-specific reactivity. Conversely, the occurrence of RT-PCR-positive but seronegative samples (12 for IgG and 8 for IgM) suggests the presence of a narrow diagnostic window period between infection and seroconversion, or variability in humoral immune responses, during which viral replication occurs in the absence of detectable antibodies.²¹ These observations are consistent with previous reports cautioning against reliance on serological antibody-based tests for diagnosing active HEV cases and reinforce the need for diagnostic methods that directly detect viral components.^{7,21,37}

These limitations of serological antibody-based assays underscore the value of our in-house antigen detection assay. While RT-PCR remains the undisputed gold standard for diagnosing viral infection, its application in routine field surveillance is often limited by high costs, technical complexity, and the need for specialized equipment and trained personnel.²² Our sandwich IgY-ELISA directly addresses these challenges. By leveraging chicken IgY technology, we have developed a diagnostic tool that combines high accuracy with practical utility. Antibody production in laying hens is a non-invasive, high-yield, and cost-effective method, circumventing the ethical concerns and financial constraints associated with mammalian antibody production.^{23,24} The rigorous *in silico* design and selection of the immunogenic peptide from the conserved ORF2 capsid region ensured the generation of specific IgY with high affinity, as confirmed by the absence of cross-reactivity with heterologous antigens like HBsAg.

The diagnostic performance of the developed sandwich IgY-ELISA represents the most significant achievement of this study. The perfect sensitivity (100%) suggests that no active infections detectable by RT-PCR were missed by our in-house sandwich IgY-ELISA test. The very high specificity (98.95%) demonstrates that false positives are exceedingly rare. The three discrepant samples (HEV-Ag negative but RT-PCR positive) likely reflect very low viral loads below

the detection limit of our in-house IgY-ELISA antigen-capture assay, a recognized scenario in which nucleic acid amplification by RT-PCR retains an advantage.^{1,7,8} Notably, no false positives were observed (all HEV-Ag-positive samples were RT-PCR-positive), confirming the high specificity of our assay. The non-significant difference ($p = 0.250$) in HEV RNA prevalence (18.6%; 95% CI: 14.5–22.7) and HEV-Ag prevalence (17.7%; 95% CI: 13.7–21.7) further validates the reliability of our assay. Indeed, this close correlation is expected, as the presence of viral capsid antigen in serum directly reflects viremia. Collectively, these findings demonstrate that our in-house sandwich IgY-ELISA test for HEV-Ag detection is a reliable and practical tool for diagnosing active infection, effectively bridging the diagnostic gap between complex molecular techniques and indirect serological antibody testing.

5. Conclusion

This study aimed to develop a sensitive, affordable, and reliable IgY-based ELISA for the efficient detection of HEV-Ag in pig serum specimens. Our findings lead to two main conclusions. First, our findings confirm a high prevalence of HEV circulation in Cameroonian pigs, representing a persistent public health risk that underscores the need for enhanced surveillance and control strategies. Second, and more importantly, we successfully developed and validated a robust, sensitive, and specific in-house IgY-ELISA for HEV-Ag detection. The assay's performance, coupled with the cost-effectiveness, scalability, and ease of production of IgY technology, makes it an ideal tool for large-scale screening programs in veterinary diagnostics and abattoir surveillance. By enabling efficient identification of viremic animals, this test can directly inform targeted control strategies, such as targeted testing of pork products or herds, thereby mitigating the risk of zoonotic HEV transmission from pigs to humans. Furthermore, it provides a viable alternative to RT-PCR for large-scale screening and diagnosing active infection in resource-constrained settings. Future research should focus on validating this assay across more diverse pig populations and investigating its potential applicability in human diagnostics.

Acknowledgments

The authors gratefully acknowledge the staff of the Bonaberi-Douala abattoir for their assistance in facilitating blood sample collection at the exsanguination point.

Funding

This research was supported by the International Center for Genetic Engineering and Biotechnology (ICGEB) (grant number: S/CMR15-06), and Ministry of Higher Education

of Cameroon under Trimester Research Modernization Allocation.

Conflict of interest

The authors declare that they have no competing interests.

Author contributions

Conceptualization: A.F. Kouam, F.N. Njyou, P.F. Moundipa

Formal analysis: A.F. Kouam, B.F.N. Simo, C.T. Zemnou, K.J. Kongnyuy, A.J. Seukep, E.M. Zeuko'o

Investigation: A.F. Kouam, P.H.N. Njitoyap, B.F.N. Simo, C.T. Zemnou, K.J. Kongnyuy, A.J. Seukep, E.M. Zeuko'o

Methodology: A.F. Kouam, P.H.N. Njitoyap, B.F.N. Simo, C.T. Zemnou, K.J. Kongnyuy, A.J. Seukep, E.M. Zeuko'o

Project administration: A.F. Kouam, F.N. Njyou, P.F. Moundipa

Software: A.F. Kouam, B.F.N. Simo

Resources: A.F. Kouam, P.H.N. Njitoyap, F.N. Njyou, P.F. Moundipa

Supervision: A.F. Kouam, P.H.N. Njitoyap, F.N. Njyou, P.F. Moundipa

Validation: A.F. Kouam, P.H.N. Njitoyap, C.T. Zemnou, A.J. Seukep, E.M. Zeuko'o, F.N. Njyou, P.F. Moundipa

Visualization: A.F. Kouam, P.H.N. Njitoyap, C.T. Zemnou, A.J. Seukep, E.M. Zeuko'o, F.N. Njyou, P.F. Moundipa

Writing—original draft: A.F. Kouam, P.H.N. Njitoyap, B.F.N. Simo

Writing—review & editing: All authors

Ethics approval and consent to participate

This study was approved by the Institutional Animal Care and Use Committee of the University of Buea (UB-IACUC N°59/2023).

Consent for publication

Not applicable.

Availability of data

All data generated or analyzed during this study are included in this published article and its supplementary information files.

References

- Lagare A, Abdoukader IK, Fall G, *et al.* Molecular epidemiology of hepatitis E virus among humans in the Niger Republic, 2017–2023. *J Clin Virol.* 2025;176:105761. doi: 10.1016/j.jcv.2025.105761
- WHO. News Room Fact Sheets Detail Hepatitis-E. World Health Organization; 2025. Available from: <https://www.who.int/news-room/fact-sheets/detail/hepatitis-e> [Last accessed on 2025 Jun 11].
- Modiyinji AF, Atsama MA, Monamele GC, Nola M, Njoum R. High seroprevalence of hepatitis E among pigs suggests an animal reservoir in Cameroon. *J Infect Dev Ctries.* 2018;12(8):676–679. doi: 10.3855/jidc.10310
- Purdy MA, Harrison TJ, Jameel S, *et al.* ICTV virus taxonomy profile: Hepeviridae. *J Gen Virol.* 2017;98(11):2645–2646. doi: 10.1099/jgv.0.000940
- Cao LC, Ha LNN, Giang TT, *et al.* Characterization of zoonotic hepatitis E virus in domestic pigs and wild boar in Vietnam: Implications for public health. *One Health.* 2024;19:100857. doi: 10.1016/j.onehlt.2024.100857
- Meng XJ. From barnyard to food table: The omnipresence of hepatitis E virus and risk for zoonotic infection and food safety. *Virus Res.* 2011;161(1):23–30. doi: 10.1016/j.virusres.2011.01.016
- Monini M, Di Bartolo I, De Sabato L, Ianiro G, Agostinelli F, Ostanello F. Hepatitis E virus (HEV) in heavy pigs in slaughterhouses of Northern Italy: Investigation of seroprevalence, viraemia, and faecal shedding. *Animals.* 2023;13(18):2942. doi: 10.3390/ani13182942
- Boxman ILA, Verhoef L, Dop PY, Vennema H, Dirks RAM, Opsteegh M. High prevalence of acute hepatitis E virus infection in pigs in Dutch slaughterhouses. *Int J Food Microbiol.* 2022;379:109830. doi: 10.1016/j.ijfoodmicro.2022.109830
- He Z, Liu D, Liu B, *et al.* Prevalence of hepatitis E virus in swine in China: A systematic review with meta-analysis (2004–2023). *Front Vet Sci.* 2025;11:1472658. doi: 10.3389/fvets.2024.1472658
- Modiyinji AF, Bigna JJ, Kenmoe S, *et al.* Epidemiology of hepatitis E virus infection in animals in Africa: A systematic review and meta-analysis. *BMC Vet Res.* 2021;17:50. doi: 10.1186/s12917-021-02749-5
- Yugo DM, Meng XJ. Hepatitis E virus: Foodborne, waterborne and zoonotic transmission. *Int J Environ Res Public Health.* 2013;10(10):4507–4533. doi: 10.3390/ijerph10104507
- Luo Q, Chen J, Zhang Y, *et al.* Viral hepatitis E: Clinical manifestations, treatment, and prevention. *Liver Res.* 2024;8(1):11–21. doi: 10.1016/j.livres.2024.01.001
- Rein DB, Stevens GA, Theaker J, Wittenborn JS, Wiersma ST. The global burden of hepatitis E virus genotypes 1 and 2 in

2005. *Hepatology*. 2012;55(4):988-997.
doi: 10.1002/hep.25505
14. Kamar N, Izopet J, Pavio N, *et al*. Hepatitis E virus infection. *Nat Rev Dis Primers*. 2017;3:17086.
doi: 10.1038/nrdp.2017.86
 15. Wu C, Wu X, Xia J. Hepatitis E virus infection during pregnancy. *Virol J*. 2020;17(1):73.
doi: 10.1186/s12985-020-01343-9
 16. Karpe YA. Processing of the hepatitis E virus ORF1 nonstructural polyprotein. *Front Virol*. 2024;3:38.
doi: 10.3389/fviro.2023.1327745
 17. Kenney SP, Meng XJ. Hepatitis E virus genome structure and replication strategy. *Cold Spring Harb Perspect Med*. 2019;9(1):a031724.
doi: 10.1101/cshperspect.a031724
 18. Wang B, Meng XJ. Structural and molecular biology of hepatitis E virus. *Comput Struct Biotechnol J*. 2021;19:1907-1916.
doi: 10.1016/j.csbj.2021.03.038
 19. Ankavay M, Montpellier C, Sayed IM, *et al*. New insights into the ORF₂ capsid protein, a key player in the hepatitis E virus lifecycle. *Sci Rep*. 2019;9:6243.
doi: 10.1038/s41598-019-42737-2
 20. Riddell MA, Li F, Anderson DA. Identification of immunodominant and conformational epitopes in the capsid protein of hepatitis E virus by using monoclonal antibodies. *J Virol*. 2000;74(17):8011-8017.
doi: 10.1128/jvi.74.17.8011-8017.2000
 21. Li Y, Yang HS, Klasse PJ, Zhao Z. The significance of antigen-antibody-binding avidity in clinical diagnosis. *Crit Rev Clin Lab Sci*. 2025;62(1):9-23.
doi: 10.1080/10408363.2024.2379286
 22. Adams NM, Leelawong M, Benton A, Quinn C, Haselton FR, Schmitz JE. COVID-19 diagnostics for resource-limited settings: Evaluation of “unextracted” qRT-PCR. *J Med Virol*. 2021;93(1):559-563.
doi: 10.1002/jmv.26328
 23. Lee L, Samardzic K, Wallach M, Frumkin LR, Mochly-Rosen D. Immunoglobulin Y for potential diagnostic and therapeutic applications in infectious diseases. *Front Immunol*. 2021;12:696003.
doi: 10.3389/fimmu.2021.696003
 24. Pereira EPV, Van Tilburg MF, Florean EOPT, Guedes MIF. Egg yolk antibodies (IgY) and their applications in human and veterinary health: A review. *Int Immunopharmacol*. 2019;73:293-303.
doi: 10.1016/j.intimp.2019.05.015
 25. Modiyinji AF, Tankeu LTA, Monamele CG, *et al*. Hepatitis E virus infections among patients with acute febrile jaundice in two regions of Cameroon: First molecular characterization of hepatitis E virus genotype 4. *PLoS One*. 2024;19(2):e0298723.
doi: 10.1371/journal.pone.0298723
 26. Locarnini SA, Yuen L. Molecular genesis of drug-resistant and vaccine-escape HBV mutants. *Antivir Ther*. 2010;15(3 Pt B):451-461.
doi: 10.3851/IMP1499
 27. Can H, Köseoğlu AE, Erkunt Alak S, *et al*. *In silico* discovery of antigenic proteins and epitopes of SARS-CoV-2 for the development of a vaccine or a diagnostic approach for COVID-19. *Sci Rep*. 2020;10:22387.
doi: 10.1038/s41598-020-79645-9
 28. Chaudhuri D, Datta J, Majumder S, Giri K. *In silico* designing of peptide based vaccine for hepatitis viruses using reverse vaccinology approach. *Infect Genet Evol*. 2020;84:104388.
doi: 10.1016/j.meegid.2020.104388
 29. Shams M, Nourmohammadi H, Asghari A, *et al*. Construction of a multi-epitope protein for human *Toxocara canis* detection: Immunoinformatics approach multi-epitope construct for *T. Canis* serodiagnosis. *Inform Med Unlocked*. 2021;26:100732.
doi: 10.1016/j.imu.2021.100732
 30. Shey RA, Ghogomu SM, Esoh KK, *et al*. *In-silico* design of a multi-epitope vaccine candidate against onchocerciasis and related filarial diseases. *Sci Rep*. 2019;9:4409.
doi: 10.1038/s41598-019-40833-x
 31. Hodek P, Trefil P, Simunek J, Hudecek J, Stiborova M. Optimized protocol of chicken antibody (IgY) purification providing electrophoretically homogenous preparations. *Int J Electrochem Sci*. 2013;8(1):113-124.
doi: 10.1016/S1452-3981(23)14006-5
 32. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. *J Biol Chem*. 1951;193(1):265-275.
 33. Bagulo H, Majekodunmi AO, Welburn SC, Bimi L. Hepatitis E seroprevalence and risk factors in humans and pig in Ghana. *BMC Infect Dis*. 2022;22:132.
doi: 10.1186/s12879-022-07127-6
 34. Mbencho MN, Hafza N, Cao LC, *et al*. Prevalence, genotype distribution, and risk factors of hepatitis E virus in blood donors, HIV patients, and pregnant women in Southwest Cameroon. *Diagn Microbiol Infect Dis*. 2025;111(4):116748.
doi: 10.1016/j.diagmicrobio.2025.116748
 35. Meta-Djoms D, Atsama-Amougou M, Ngamaleu MR, *et al*.

Molecular surveillance of hepatitis E virus in wastewater in Yaoundé, Cameroon. *PLoS One*. 2025;20(8):e0322765.

doi: 10.1371/journal.pone.0322765

36. Modiyinji AF, Sanding GMAM, Atsama MA, Monamele CG, Nola M, Njouom R. Serological and molecular investigation of hepatitis E virus in pigs reservoirs from Cameroon reveals

elevated seroprevalence and presence of genotype 3. *PLoS One*. 2020;15(2):e0229073.

doi: 10.1371/journal.pone.0229073

37. Landry ML. Immunoglobulin M for acute infection: True or False? *Clin Vaccine Immunol*. 2016;23(7):540-545.

doi: 10.1128/CVI.00211-16