



Detection and molecular characterization of a first isolate of rabbit haemorrhagic disease virus in Nigeria

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Abstract

Rabbit haemorrhagic disease virus (RHDV) was recovered from necropsied rabbits that died during an outbreak characterized by epistaxis, incoordination, paralysis, and multi-organ haemorrhages in Ilorin, Nigeria. The haemagglutination test (HA) and RT-PCR assay targeted against a fragment of the RHDV VP60 gene were performed on liver, spleen, and kidney homogenates; faeces; and urine obtained from the rabbits. Amplicons were purified, sequenced, and phylogenetically analysed. The liver homogenates yielded the highest HA titres while RT-PCR of liver, spleen, and kidneys yielded the expected 1252 bp band. Sequence and phylogenetic analyses revealed that the Nigerian RHDV strain (RHDV/NGR/ILN/001) was 98.57%, 97.95%, and 96.70% homologous with RHDV2 (RHDVGI.2) strains from the Netherlands, Germany, and France, respectively. RHDV/NGR/ILN/001 induced tracheal, intestinal, and mediastinal lymph node haemorrhages, pulmonary oedema and congestion, and enlarged, necrotic liver in experimentally inoculated rabbits. The implications of this study, which is the first report of RHDV in Nigeria, are discussed.

Keywords Rabbit haemorrhagic disease virus · Haemagglutination test · RT-PCR · Sequencing and phylogenetic analysis · Nigeria

Introduction

Since the first report of rabbit haemorrhagic disease (RHD) in the Jiangsu Province of China in 1984 (Liu et al. 1984), it has spread rapidly to countries of Europe, the Americas, Asia, and the Middle East, becoming endemic in few years (Le Gall-Reculé et al. 2003; Forrester et al. 2006; Farnos et al. 2007; Kerr et al. 2009; Abrantes et al. 2012). The disease manifests as a highly contagious, acute or peracute and rapidly fatal condition among domestic and wild rabbits (*Oryctolagus cuniculus*) causing severe haemorrhages in multiple organs, mortality up to 90%, and consequent huge economic losses in the rabbit meat and fur production industries (Villafuerte et al. 1995; Delibes-Mateos et al. 2007, 2008; World Organisation for Animal Health (OIE) 2019a). Apart from the fact that infection with RHDV induces massive liver cell death via necrosis and apoptosis, the pathogenesis of RHD also includes apoptosis of granulocytes and peripheral blood lymphocytes, as well as direct systemic haemorrhagic diathesis and disseminated intravascular coagulation. Additionally, liver-specific microRNAs (ocu-miR-122-5p, ocu-miR-155-5p, and ocu-

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miR-16b-5p) have been reported to play significant roles in the pathogenesis of RHD as their levels in experimentally infected rabbits correlated with hepatic inflammation and dysfunction (Hukowska-Szematowicz et al. 2020).

Rabbit haemorrhagic disease is caused by the RHD virus (RHDV), which is a non-enveloped single-stranded, positive-sense RNA virus belonging to the family *Caliciviridae* and genus *Lagovirus* (Meyers et al. 1991). The virus genome possesses two open reading frames (ORF): ORF1 (nucleotides 10–7044) that encodes a large polyprotein that is post-translationally cleaved to produce the major capsid protein (VP60) and the non-structural proteins, including the RNA-dependent RNA polymerase (RdRp), and ORF2 (nucleotides 7025–7378) encoding a minor structural protein (VP10) (Meyers et al. 1991; Dalton et al. 2015). Phylogenetic analyses of pathogenic RHDV strains reveal that three distinct circulating groups exist, including the classical RHDV (comprising genogroups G1–G5), the RHDVa (genogroup G6), and the newly emerged RHDV2 (Le Gall-Recule et al. 2003; Muller et al. 2009; Alda et al. 2010; World Organisation for Animal Health (OIE) 2019a). The RHDV and RHDVa are more closely related to each other phylogenetically but their capsid protein (VP60) differs substantially in nucleotide diversity from that of RHDV2. Recently, a unified classification system and nomenclature have been proposed for lagoviruses due to their diversity and continuous discovery of new members (Pendur et al. 2017).

Generally, oral, nasal, and conjunctival routes of transmission have been identified for RHDV. It can be transmitted through direct contact with infected rabbits, indirect contact with contaminated fomites (feed, water, clothing, bedding, shoes, cages, and equipment), and mechanically through mosquito and flea vectors (Cooke 2002; World Organisation for Animal Health (OIE) 2019a). Contaminated rabbit meat and by-products such as fur are also known to be infectious (Abrantes et al. 2012).

According to Gleeson and Petritz (2020), a presumptive diagnosis of RHD can often be made based on clinical presentation, infection pattern within a population, and post-mortem lesions while definitive diagnosis requires serologic and molecular methods of viral detection since most caliciviruses cannot be grown in cell culture. Laboratory diagnosis of RHDV involves the use of the haemagglutination (HA) test (based on its ability to agglutinate human type O red blood cells), sandwich enzyme-linked immunosorbent assay (ELISA), immune electron microscopy (EM), immunostaining and Western blotting for virus detection, haemagglutination inhibition (HI) test and ELISA for antibody detection, and viral RNA amplification by the reverse transcriptase-polymerase chain reaction (RT-PCR) (Capucci et al. 1991; World Organisation for Animal Health (OIE) 2019a). Since attempts to infect routine laboratory animals or chick embryos were unsuccessful (Wang et al. 1986; Buonavoglia et al. 1988; Pages 1989), inoculation of the

natural rabbit hosts remains the only way in which RHDV can be isolated, propagated, and titrated (World Organisation for Animal Health (OIE) 2019a).

Outbreaks of rabbit haemorrhagic disease have only been recorded in few African countries including Egypt (Ghanem and Ismail 1992; Magouz et al. 2019), the Republic of Benin (Kpodekon and Alogninouwa 1998; World Organisation for Animal Health (OIE) 2015), Tunisia (Bouslama et al. 1996; Rahali et al. 2019), and Morocco (Lopes et al. 2019), with no previous report from Nigeria. This study presents the findings of an investigation aimed at providing a confirmatory diagnosis of the first outbreak of haemorrhagic disease among domesticated rabbits in Nigeria.

Material and methods

An outbreak of a fulminating disease in rabbits was reported to the Ilorin Zonal Veterinary Clinic and Diagnosis Centre and Veterinary Teaching Hospital (VTH), University of Ilorin, Ilorin, Kwara State, Nigeria, between June and September 2020. Affected farmers reported that the animals were raised on commercially produced feed under intensive management. The disease, which was characterized in the rabbits by pale conjunctiva, epistaxis, incoordination, and occasional paralysis, caused 80–100% mortality in the affected rabbitries. Necropsy was performed on 30 rabbit carcasses submitted to the VTH during the period and tissue (liver, spleen, kidney), faeces, and urine samples were collected from 10 of them for confirmatory laboratory diagnosis. All the 30 rabbits were adults of both sexes and belonged to the Hyla, Angora, and Chinchilla breeds.

Detection of RHDV protein by haemagglutination test

For detection of the haemagglutinating property of RHDV, 10% suspensions of harvested tissue homogenates were prepared in phosphate-buffered saline (PBS, pH 6.8) and clarified by centrifugation at 4000 rpm for 5 min. The supernatants were decanted into sterile 2-ml cryovials for storage at -20 °C. The HA test was performed using 0.75% washed human type O red blood cells (RBC) according to the World Organization for Animal Health (OIE) protocol (World Organisation for Animal Health (OIE) 2012). An initial 1:10 dilution of each supernatant in PBS was made. Thereafter, 50- μ l PBS was dispensed into all the wells of a U-bottom microtitre plate followed by twofold serial dilution of 50- μ l clarified supernatant of each tissue suspension, faeces, and urine sample. Thereafter, 50- μ l washed RBC was dispensed into all the wells and the plate tapped gently before incubation at 4 °C for 1 h. Each sample was tested in three replicates. The haemagglutination titre was taken as the reciprocal of the highest dilution producing complete agglutination of erythrocytes. Samples with titres > 1:160 were considered positive.

RNA extraction

RNA was extracted from clarified supernatants of all samples using the Viral RNA+DNA preparation kit (Jena Bioscience, Germany). Briefly, 150 µl of supernatant was dispensed into a 1.5-ml Eppendorf tube followed by the addition of 300-µl lysis buffer. The mixture was vortexed for 15 s and left to incubate at room temperature for 10 min before 300-µl binding buffer was added and subsequently vortexed. Thereafter, all the lysate was dispensed into a spin column placed in a 2-ml collection tube and this was spun at 13,000g for 1 min. The spin column was then washed with 500-µl Washing Buffer A, centrifuged at 13,000g for 1 min and the flow-through in the collection tube discarded. The washing step was again repeated with 500-µl Washing Buffer B. After discarding the flow-through, the spin column was placed into the collection tube and centrifuged again at 13,000g for 1 min to ensure complete removal of ethanol residue. Subsequently, the spin column was placed into a new 1.5-ml Eppendorf tube and allowed to incubate for 1 min at room temperature. The RNA was then eluted at 13,000g for 1 min using 50-µl elution buffer.

cDNA synthesis

Complementary DNA (cDNA) synthesis was carried out in two steps using the SCRIPT cDNA synthesis kit (Jena Bioscience, Germany). Briefly, a mixture of 8.5-µl RNA extract, 0.5-µl random hexamer, and 1-µl RNase-free water was prepared and incubated for 5 min at 65°C. Thereafter, a mixture comprising 1-µl DTT, 1-µl dNTPs, 0.5-µl RNase inhibitor, 0.5-µl reverse transcriptase, 4-µl buffer solution, and 3-µl RNase-free water was prepared and added to the first mixture. The final 20-µl mixture was incubated at 42°C for 10 min and then at 50°C for 40 min.

Polymerase chain reaction and gel electrophoresis

PCR amplification was performed with a total volume of 40-µl reaction containing 10-µl Red Load Taq master mix (Jena Bioscience, GmbH), 0.8 µl of each primer, 5-µl DNA, and 23.4-µl RNase-free water. The primers RHDV-NG-F (5'-CCCGCTAACCCTATGACTTGG-3') and RHDV-NG-R (5'-AACCGTGTCTAGTGTCTGCC-3') were designed to amplify a 1252 base pair (bp) fragment (nt 697–1948) in the RHDV capsid protein (VP60) gene. Thermal cycling was done using the MiniAmp™ Plus thermal cycler (Thermo Fisher Scientific, USA) as follows: 90 °C for 50 s, followed by 35 cycles of 94°C for 30 s, 57°C for 30 s, and 72°C for 45 s. The final extension was for 2 min at 72°C. The PCR amplicons were loaded on a 1.8% agarose gel stained with Gel Red in 1X Tris-acetate EDTA (TAE) buffer and visualized using the CyFox® live imaging and gel electrophoresis device (Sysmex Partec GmbH, Germany).

Sequencing and phylogenetic analysis

The amplicons were cleaned up using ExoSAP-IT Fast reagent (Thermo Fisher Scientific, USA). Five microlitres of amplicon was mixed with 2 µl of the reagent and incubated at 37°C for 7 min and then at 80°C for 7 min. Sequencing was performed using the BigDye® Terminator v3.1 cycle sequencing kit (Perkin-Elmer, Foster City, CA) and the product was analyzed using an ABI 3130xl Genetic Analyzer (Applied Biosystems, USA). The sequences generated were assembled into a single contig using DNASTAR Lasergene v7.0 and aligned with 29 published RHDV sequences (14 GI.1, 12 GI.2, and 3 GI.3 strains) downloaded from the GenBank database (<https://www.ncbi.nlm.nih.gov/GenBank>) using BLASTn with rabbit haemorrhagic disease virus as query. Multiple sequence alignment was performed to establish similarities with the reference GenBank sequences using the MAFFT v.7 (Nakamura et al. 2018) and BioEdit software (Informer Technologies, USA), while phylogenetic analysis was conducted with MEGA X (Kumar et al. 2018), applying the maximum likelihood method and Tamura-Nei model. The bootstrap consensus tree was inferred from 1000 replicates based on the general time-reversible (GTR) substitution model with gamma distribution.

Experimental infection

In order to further confirm the presumptive diagnosis of RHD made based on the clinical signs and lesions observed in the necropsied rabbits, three female Chinchilla breed rabbits of 2.5 kg (rabbit A), 1.5 kg (rabbit B), and 2 kg (rabbit C) body weights, respectively, were used for pathogenicity studies. The rabbits, which were obtained from a farm with no history of RHD, were stabilised for 72 h and fed with *Tridax procumbens* ("coat buttons") and water. A 20% (w/v) suspension of pooled liver and spleen homogenates harvested from rabbits that died during the outbreak was prepared in sterile normal saline containing 50 µg/ml gentamycin, 2 mg/ml streptomycin, 2000 units/ml penicillin, and 1000 units/ml amphotericin B. The suspension was then centrifuged at 4000 rpm for 10 min. Subsequently, rabbit A was inoculated intramuscularly with 1 ml of the clarified supernatant and rabbit B received 1 ml of the supernatant intraperitoneally, while rabbit C (control) was inoculated intramuscularly with 1 ml of normal saline containing the above-mentioned antibiotics and antifungal medication. The rabbits were then placed in separate cages and observed every 6 h for clinical signs. The post-mortem examination was performed on the experimental rabbits immediately after death. Liver and spleen samples from the dead rabbits were then extracted and processed for RT-PCR using the same procedures and primers described above.

Results

Necropsy findings in rabbits from outbreak

Post-mortem findings in some of the rabbit carcasses revealed epistaxis with serosanguineous fluid oozing out of the nostrils onto the upper lip and face (Fig. 1a), large straw-coloured mucoid exudate plugging the lower 1/3rd of the tracheal lumen down to the bifurcation of the trachea into the lungs, diffuse ecchymotic haemorrhages of the tracheal mucosae measuring about 10 mm in diameter, and moderate congestion (Fig. 1b–d). In some of the rabbits, especially those with haemorrhagic tracheae, the lungs were severely oedematous and congested

with multifocal patchy consolidation (Fig. 1b and e), while the livers were enlarged and friable with rough edges and diffuse pale areas of necrosis (Fig. 1e). The hearts were globose with thickened ventricles and multifocal ecchymoses (Fig. 1f). In addition, there were haemorrhages in the small intestinal mucosa especially in the proximal 2/3rd, ballooning of the large intestines, especially the caeca, with moderate intraluminal faecal material (Fig. 1g), and ecchymotic haemorrhages on the mediastinal lymph nodes (Fig. 1h). The kidneys were enlarged with some being pale on their cut surfaces while others were severely haemorrhagic when cut (Fig. 1i). Some of the rabbits had distended urinary bladders which contained yellow turbid urine (Fig. 1j). Histopathologic examination

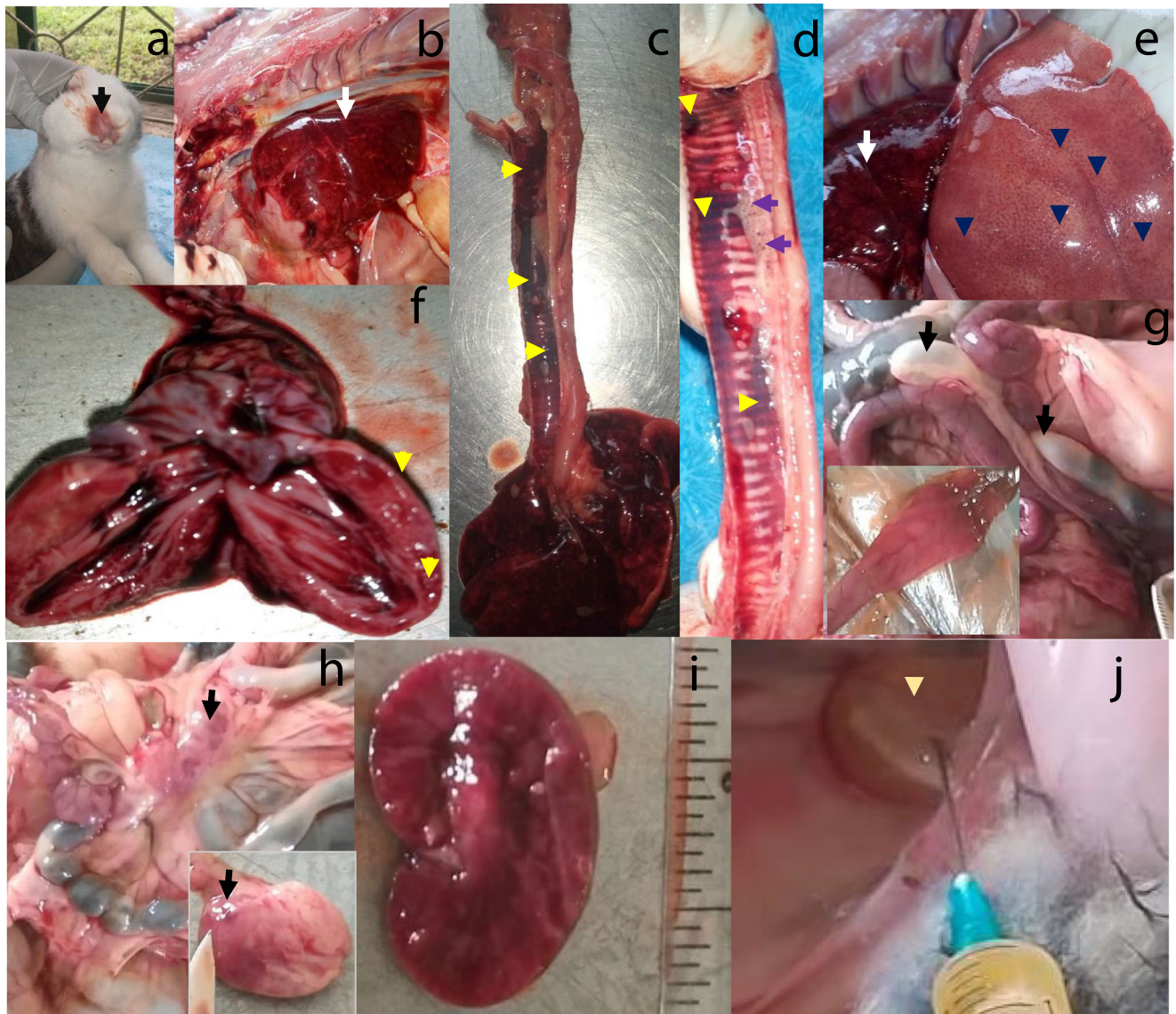


Fig. 1 Gross pathologic features of RHD in infected rabbits showing epistaxis (a); tracheal lumen with diffuse haemorrhages (b) and frothy exudate (purple arrows) (c); severely congested left lung (white arrow) (d); enlarged liver with rough edges and focal areas of necrosis (blue arrows) (e); globose heart with thickened ventricle (yellow arrows) (f);

ballooned large intestine with prominent blood vessels and haemorrhage in the proximal 2/3rd of the small intestine (g); ecchymotic haemorrhages on the mediastinal lymph nodes (black arrow) (h); enlarged kidney with multifocal areas of necrosis (i); distended bladder filled with yellow turbid urine (j)

revealed severe congestion, oedema, and eosinophilic materials in the lung alveoli (Fig. 2a); severe congestion, periportal degeneration, and mononuclear cell infiltration in the liver (Fig. 2b and c); and glomerular congestion and haemorrhage in the capsular space with tubular nephrosis (Fig. 2d).

RHDV haemagglutinating activity

The liver homogenates yielded the highest RHDV HA titres followed by spleen and kidney homogenates (Table 1). Eight of the 10 liver samples tested were positive for HA activity with titres ranging from 1:320 to 1:40960. For spleen and kidney samples, eight and nine samples were positive with HA titre ranges of 1:320 and 1:640, respectively.

Reverse transcriptase-polymerase chain reaction

A specific 1252-bp band corresponding to a fragment of the VP60 gene of RHDV was amplified by RT-PCR in 7 of the 10 rabbit liver, spleen, and kidney homogenates tested (Table 1).

Sequencing and phylogenetic analysis

The sequence of the Nigerian RHDV strain, designated RHDV/NGR/ILN/001, was submitted to the GenBank database with the accession number MT996357. BLASTn analysis demonstrated that it was 98.57%, 97.95%, and 97.00% homologous at the nucleotide sequence level with RHDV GI.2 (RHDV2) strains reported in the Netherlands (MN061492.1), Germany (MN901451.1), and France (MN786321.1), respectively. Alignment of the deduced amino acid sequences of RHDV/NGR/ILN/001 and these three reference strains revealed 99.73%, 99.46%, and 99.46% percentage identities, respectively, with the Nigerian isolate differing from the other three at a maximum of two positions (Table 2). In addition, phylogenetic analysis of the nucleotide sequences of the 29 RHDV strains showed that RHDV/NGR/ILN/001 clustered with these three strains (Fig. 3).

Clinicopathological findings in experimental rabbits

Rabbit A showed signs of dullness 18 h post-inoculation (p.i.), became recumbent by 20 h p.i., and died by 23 h p.i. while

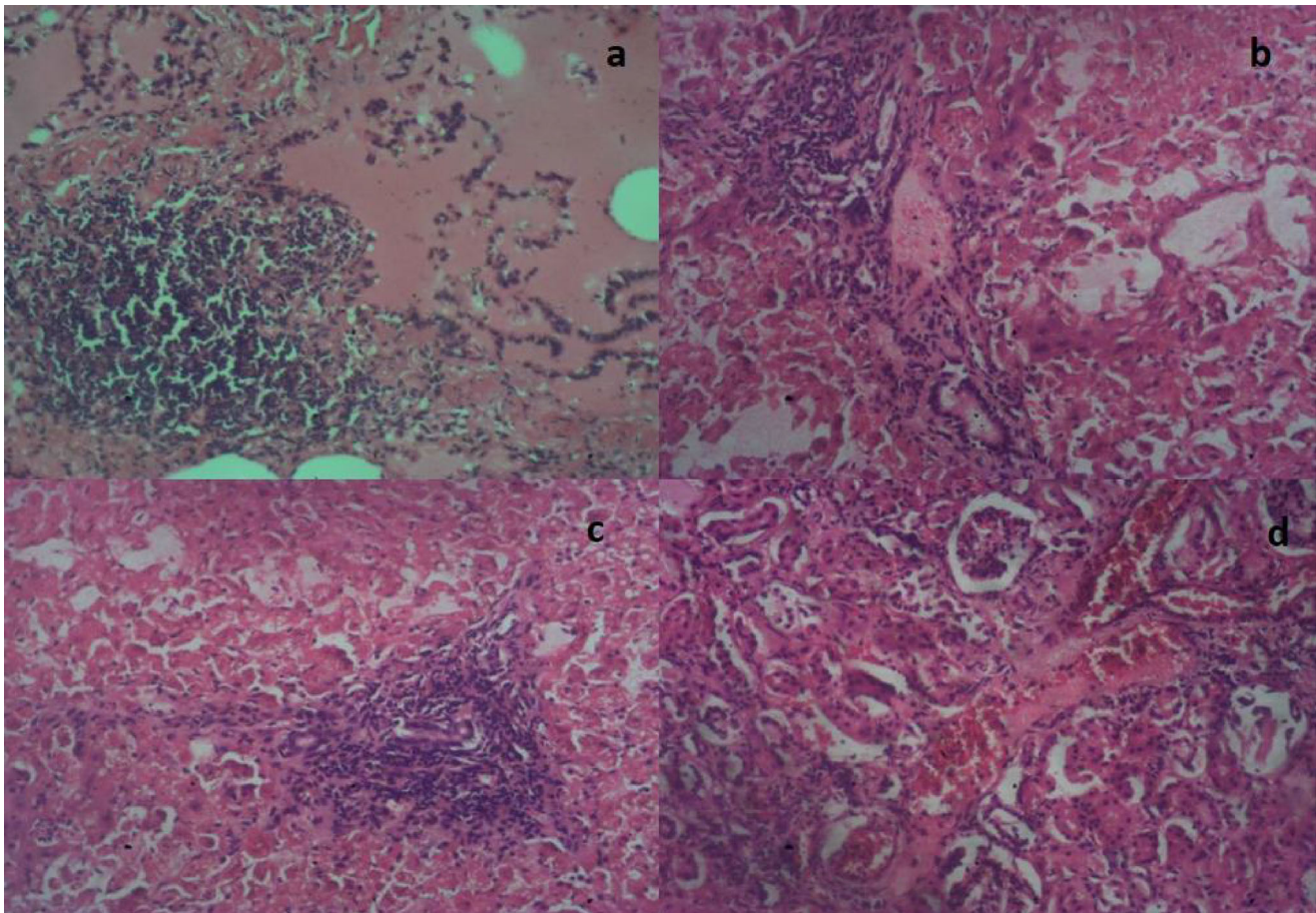


Fig. 2 Histopathologic features of RHD in infected rabbits showing: lungs with severe congestion, oedema, and eosinophilic materials in the alveoli (a); liver showing periportal hyper-eosinophilic materials and

severe congestion (b); liver with periportal degeneration and mononuclear cell infiltration (c); kidney characterized by glomerular congestion and haemorrhage in the capsular space and tubules, and tubular nephrosis (d)

Table 1 Samples positive for RHDV using HA test and RT-PCR

Sample	HA positive (%)	HA titre range	Geometric mean titer (Log ₂)	RT-PCR positive (%)
Liver	8/10 (80)	1:320–1:40960	71.16	7/10 (70)
Spleen	8/10 (80)	1:320–1:1280	52.32	7/10 (70)
Kidney	9/10 (90)	1:320–1:640	51.80	7/10 (70)
Faeces	10/10 (100)	1: 5120	7.0	nt
Urine (undiluted)	0/10 (0)	-	-	nt

nt, not tested

rabbit B died after 48 h of infection without significant signs. Physical examination revealed seromucous discharge from the anus (1/2). Pathological lesions observed were similar to those seen in the tracheal, lungs, and liver of carcasses obtained from the outbreak. Additionally, severe ecchymotic haemorrhages were observed along the whole length of the intestinal tract with petechial haemorrhages on the mediastinal lymph nodes (2/2). Histopathologic features were also similar in these organs. RT-PCR testing of liver and spleen RNA extracts from the experimentally infected rabbits yielded RHDV-specific 1252-bp bands following agarose gel electrophoresis (data not shown).

Discussion

In the past three decades, rabbits have been recognized as tools for poverty alleviation and food security in developing countries (Lukefahr and Cheeke 1991; Owen et al. 2005; Oseni and Lukefahr 2014). Recently, there has been a progressive intensification of rabbit rearing and increasing demand for rabbit meat in Nigeria. This might be attributable to their high protein content, low investment in terms of housing, feeding, and general management, their rapidly reproducing and highly prolific nature, and attendant early benefits. However, the threat of RHD to this evolving livestock sector in Nigeria had remained potent with earlier reports of the disease in other African countries including Egypt, Republic of Benin, Tunisia,

and Morocco (Ghanem and Ismail 1992; Magouz et al. 2019; Kpodekon and Alogninouwa 1998; Bouslama et al. 1996; Rahali et al. 2019; Lopes et al. 2019). In this study, which to our knowledge is the first-record of detection and molecular characterization of RHDV in Nigeria, characteristic disease-associated clinical signs and lesions as well as the findings of HA test, RT-PCR, and sequencing and phylogenetic analyses have confirmed RHDV as the cause of an outbreak of haemorrhagic disease in rabbits in Ilorin, Kwara State.

The HA test results, which showed that our isolate (RHDV/NGR/ILN/001) was able to agglutinate human type O RBCs, revealed that the liver had the highest viral titre although the virus was also detectable in the spleen, kidney, and faeces. This observation is consistent with previous reports (Ahmad et al. 2011; Magouz et al. 2019; World Organisation for Animal Health (OIE) 2019a) indicating that the liver contains the highest concentrations of RHDV by the HA test, thus suggesting that hepatocytes are the preferred sites for RHDV replication. It has been noted that testing for hemagglutinating activity in liver samples is a major cornerstone for rapid diagnosis of RHDV outbreaks in rabbits (Liu et al. 1984; World Organisation for Animal Health (OIE) 2019a), since it is an easy and readily available technique. Moreover, the detection of RHDV antigen in the liver has been reported to be possible within the first hours post-infection (p.i.) (Jung et al. 2000; Prieto et al. 2000; Kimura et al. 2001) and can reach peak levels in ≤ 2 days p.i. (Gelmetti et al. 1998; Jung et al. 2000).

Table 2 Nucleotide and amino acid differences between Nigerian and selected reference RHDV2 (GI.2) strains

Reference RHDV2 (GI.2) strain (accession number/country)	RHDV/NGR/ILN/001 (nt 790-1908)		
	No. of nucleotide differences	No. of amino acid differences	Amino acid position (substitution)
MN061492.1/ Netherlands	16	1	368 (aspartic acid → glycine)
MN901451.1/ Germany	22	2	338 (threonine → isoleucine) and 368 (aspartic acid → glycine)
MN786321.1/ France	37	2	266 (lysine → arginine) and 368 (aspartic acid → glycine)

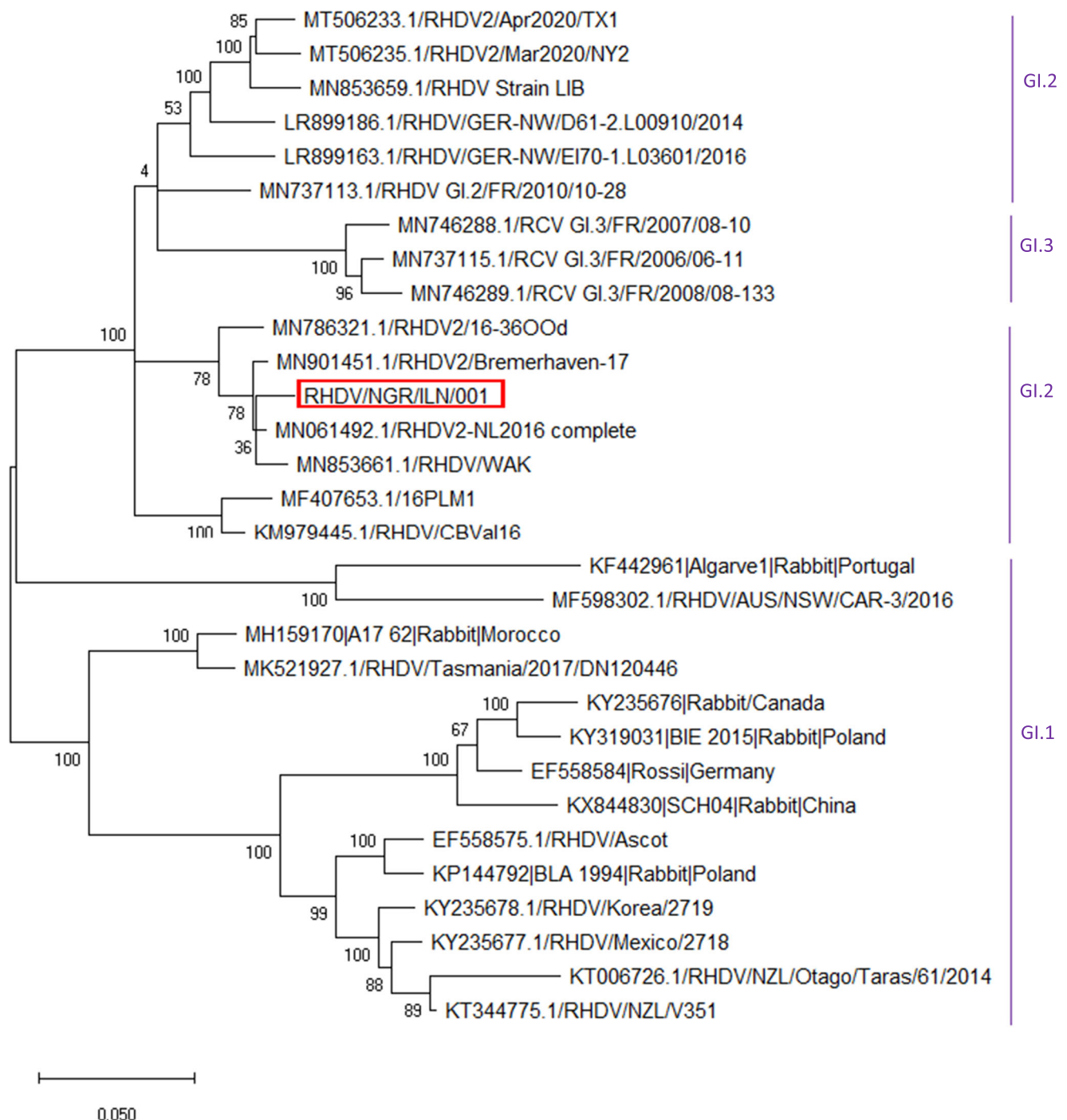


Fig. 3 Phylogenetic relationship among Nigerian and reference RHDV strains based on nucleotide sequence of a fragment of the VP60 gene

It is noteworthy that the clinical history of epistaxis, incoordination, and paralysis presented by the rabbit farmers in the current outbreak as well as dullness and recumbency observed in the experimentally infected rabbits are consistent with the clinical signs of RHD as previously described (Abrantes et al. 2012; Brabb and Di Giacomo 2012; Kerr and Donnelly 2013). In addition, the gross and histopathological lesions seen in necropsied rabbits in this investigation, including necrosis, congestion, and periportal inflammation of the liver,

oedematous and congested lungs, severely congested tracheae with ecchymotic haemorrhages and frothy fluid, haemorrhages in the intestines and mediastinal lymph nodes, glomerular congestion and nephrosis, and distended urinary bladder with yellow turbid urine are characteristic of RHD as reported by other workers (Abrantes et al. 2012; Brabb and Di Giacomo 2012; Embury-Hyatt et al. 2012; Kerr and Donnelly 2013; Magouz et al. 2019).

Our study genetically confirmed that the current outbreak was caused by RHDV2 as BLAST search, multiple sequence alignment and phylogenetic analysis based on a fragment of the VP60 capsid gene revealed a high level of identity (96.70–98.57%) between RHDV/NGR/ILN/001 and three other RHDV2 strains from the Netherlands, Germany, and France (NCBI, 2020). Furthermore, alignment of the deduced amino acid sequences of RHDV/NGR/ILN/001 and the three reference strains demonstrated that this fragment of the RHDV VP60 gene appeared to be highly conserved as the Nigerian strain exhibited a maximum diversity of 0.54% from the others.

Since vaccination against RHDV is not currently practiced in Nigeria, it is possible that the virus was introduced into the country through importation of infected rabbits or contaminated fomites. Incidentally, the closest African country to Nigeria with a history of RHD is the Republic of Benin which shares international border with Kwara State where the current outbreak occurred. Considering the illegal transborder movement of livestock from neighbouring West African countries into Nigeria, it is likely that RHDV-infected rabbits could have been imported into the country without being detected. This underscores the need for the establishment of well-equipped diagnostic facilities at the country's ports of entry where exotic diseases like RHD and other endemic and emerging infectious diseases of livestock can be rapidly detected and affected animals quarantined.

In order to prevent the spread of RHD into clean rabbitries across Nigeria and thus sustain the growth of the evolving rabbit meat industry, we advise that rabbit farmers implement biosecurity measures including restriction of access, sanitation, and disinfection of cages and all equipment entering or leaving the rabbitry. Additionally, newly acquired rabbits should be quarantined for at least 1 month and screened for anti-RHDV antibodies, while colonies with disease should be depopulated since surviving rabbits have been reported to shed virus for at least a month (Gregg et al. 1991). Continuous surveillance for RHD as well as the prophylactic use of RHDV vaccines in breeder rabbits (World Organisation for Animal Health (OIE) 2019a) can also be adopted.

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Data and materials availability The authors declare that all information as regards this study is available online for public view.

Code availability Not applicable

Author contribution Not applicable

Declarations

Ethics approval All relevant international, national, and/or institutional guidelines for the care and use of animals were duly followed (World Organisation for Animal Health (OIE) 2019b). In addition, ethical clearance for this study was obtained from the Ethics Committee of the Kwara State Ministry of Agriculture and Rural Development (VKW-714/1/182)

Consent to participate The consent of all rabbit owners and the appropriate government agency was sorted before this study was carried out.

Consent for publication Consent on publication was sorted and with approval from the appropriate government agency.

Conflict of interest The authors declare no competing interests.

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