NIGERIAN VETERINARY JOURNAL
ISSN 0331-3026
Nig. Vet. J., June 2025 Vol 46(2):19-32.
https://dx.doi.org/10.4314/nvj.v46i2.3 ORIGINAL ARITICLE

Serological and Molecular Surveillance of Rabbit Haemorrhagic Disease in Nigeria

JUDITH DIZOT BAKAM*[1], BITRUS INUWA[1], OLUMUYIWA OYEKAN[1], JESSE JONATHAN[1], OLAYINKA ASALA[1], SHITTU ISMAILA[1], TAIWO OLASOJU[2], CLEMENT MESEKO[1].

1. Regional Laboratory for Animal Influenza & Transboundary Animal Diseases, National Veterinary Research Institute, Vom, Nigeria

2. Federal Department of Veterinary Service and Pest Control, Ministry of Agriculture and Food Security

*Corresponding authors**:** judithbakam@gmail.com clementmeseko@gmail.com

SUMMARY

Rabbit Haemorrhagic Disease (RHD) is caused by Rabbit Haemorrhagic Disease Virus (RHDV) and has global mortality rate of 80-100% in rabbits. In Nigeria, it was first reported in Kwara State in 2020 despite speculations that RHDV has been seen earlier. Rabbit meat is a significant source of protein and the production venture confers economic stability especially in developing countries including Nigeria; an emerging novel infection like RHD therefore pose a challenge to food security. The aim of this investigation is to determine the serological and molecular evidence of RHDV circulation in the Nigeria. A total of 3832 sera and 119 tissue samples were collected across 25 states in 6 geopolitical zones in Nigeria within 4 weeks period from November to December 2020. This samples were analyzed using the enzyme-linked immunosorbent and Real time reverse transcriptase assays. A total seroprevalence of 2.82 % (108/3832) was recorded. The RHDV antibodies were most frequently found in the rabbit population in Ekiti State (11.74%), whereas Akwa Ibom State has the lowest prevalence (0.51%). South West had a prevalence of 6.58 % while to South-South had 0.66 %. All tissue samples tested negative for RHDV2 VP60 gene. Our findings show that RHDV antibody is present in the rabbit population across all of Nigeria's geopolitical zones. Vaccinations should be encouraged to prevent infection and manage the spread of RHDV and lessen the anticipated socioeconomic effects of rabbit hemorrhagic disease.

Keywords: Serology, PCR, Rabbit Hemorrhagic Disease, RHDV2

INTRODUCTION

In Nigeria, rabbit farming is mostly traditional, and it might be important for achieving protein intake goals (Abu, 1995), food security, as well as enhance additional revenue by rural residents (Lukefahr, 2007, FAO 2001). Unlike poultry farms that are widely spread, rabbitries are fewer and between 3.4 and 5.2% of the Nigeria population raise rabbits mostly for food, as pets, laboratory research purposes (Onifade *et al*., 1999). Like poultry, rabbitry is faced with infectious disease challenges (Meseko *et al*., 2023). Diseases, such as RHD, have been identified as one of the biggest challenge of rabbit husbandry around the world (Abrantes *et al.,* 2012). Emerging and re-emerging infectious diseases like RHD may be associated with several human factors including changes in husbandry practices towards intensifications. These human activities coupled with environmental drivers such as temperature, humidity and many more are involved in the disease emergence (Otu *et al*., 2024). Rabbit haemorrhagic disease is an acute, contagious disease with incubation period of 1-6 days and a deadly form of viral hepatitis that affects mainly domestic and wild rabbits (Bárcena *et al.,* 2015). Lethargy, anorexia, paralysis, and various organ hemorrhages are symptoms of the disease (Tunon *et al.,* 2003). However, the disease's clinical manifestation can occasionally take an acute form with mortality occurring within 48 h and a sub-acute form with mortality occurring within two weeks (Rosell *et al.,* 2019). Rabbit haemorrhagic disease virus is a member of the family *Caliciviridae and* genus *Lagovirus* (Ohlinger *et al.,* 1990). It is a non-enveloped, single stranded and a positive sense RNA organized into two slightly overlapping open reading frames (ORF). Its genome comprise of approximately 7.4 kb and exist in three different strains RHDV, RHDVa, RHDb which is differentiated based on phylogenetic and antigenic properties (Le Pendu *et al.,* 2017, Mahar *et al*., 2018, Erfan *et al*., 2020). Rabbit haemorrhagic disease virus can be spread indirectly by vectors, contaminated equipment and directly by contact with infected animals, carcasses, bodily fluids (McColl *et al.,* 2002, Calvete *et al.,* 2019).

The RHD virus highly virulent, and outbreaks of the disease has caused the death of millions of wild and farm rabbits in China, Europe, Australia, Africa, and other places (Ambagala *et al.,* 2002, Mahar *et al.,* 2018, Rouco *et al.,* 2018, Hu *et al.,* 2021). Consequently, there is a need to raise awareness about detection and management throughout west Africa, particularly in Nigeria, where rabbit farming has a significant potential due to the country's enormous population and rising protein need (Inyeinyang *et al.,* 2013). The isolated RHDV2 from outbreak in Ilorin, Nigeria, has strain (RHDV/NGR/ILN/001) similar to RHDV2 strains from the Netherlands, Germany, and France by phylogenetic analysis of the isolates (Daodu *et al.,* 2021). Through vaccination, the disease can simply be controlled indirectly. Though RHDV cannot be maintained in cell culture, it has been demonstrated that insect cells can modify the recombinant VP60 protein to produce virus-like particles (VLPs) that are identical to infectious RHD virions (Qi *et al.,* 2020, Dalton *et al.,* 2021). Rabbit haemorrhagic disease virus pathogenic

strains receive protective immunity from a non-pathogenic strain that is genetically linked to RHDV (Capucci *et al.,* 1996, Capucci *et al.,* 1997) and it was reported that its presence in the UK helped shield the rabbit population from a virulent strain of RHDV (White *et al.,* 2004). The pathogenic nature of RHDV2 and its capacity to spread quickly throughout domestic, wild, and previously immunized populations of rabbits have generated severe concerns about the epidemiological underpinning of RHDV in Nigeria (Hänske *et al.,* 2021). Consequently, this study represents an active step toward better understanding the prevalence and rate of RHD spread in Nigeria in order to design control strategies because of rabbitry economic and food security implications.

MATERIALS AND METHODS

Study Area

This study was conducted in twenty-five states of the six Geopolitical zones (GZ) of Nigeria (Table 1). North-east GZ: Adamawa, Bauchi, Taraba and Yobe states. North-central GZ: Benue, Niger, Nasarawa and Plateau states. North-west GZ: Kano and Kaduna states. South-east GZ: Abia, Ebonyi, Enugu and Imo states. South-west GZ: Ekiti, Lagos, Kwara, Ogun, Ondo and Oyo states and the South-south GZ which included Akwa-Ibom, Cross-River, Edo, Delta and Rivers State.

Study Design/Sample Collection

In a cross-sectional study carried out within a 4-weeks period in November to December 2020. 3-5 ml blood samples were collected via the marginal ear vein from apparently healthy rabbits across the 25 (Table 1) states in the six GZs of the study area through a convenient sampling method. This was based on the availability of rabbits at the time of collection. The blood samples were then transferred into plain clotting tubes and kept in ice box and were transported to the Regional Laboratory for Animal Influenza and Transboundary Animal Disease, National Veterinary Research Institute (NVRI), Vom, Nigeria. In the laboratory, the samples were centrifuged at 10,000 xg for 5 min to allow for proper separation of serum from the clotted blood. Clear separated sera were then decanted into appropriately labelled 2 mL cryovial tubes and stored at -20°C before serological analysis. At the time of blood sampling from the rabbitries across the states, 119 tissues (liver) were also collected from apparently healthy rabbits. These were transported alongside the blood sample. In the laboratory, tissue samples were homogenized in phosphate buffered saline (PBS) to a final concentration of 20% (w/v). The homogenates were vortexed and centrifuged for 5 min at 3000 xg. Supernatants were decanted into properly labeled tubes and kept at -80°C freezer until viral RNA extraction. All samples (sera and tissues) collected in the study were irrespective of age, breed and sex of the rabbits.

RHDV Serological Assay

Antibodies to RHDV were tested using indirect enzyme-linked immunosorbent assay (ELISA) from (INGEZIM RHDV R.17.RHD.K1, Inmunologia Y Genetica Aplicada, S.A. Madrid, Spain). The antigen is fixed in a solid support (polystyrene plate). The antigen is recombinant protein from RHDV obtained using baculovirus system of expression. The serum sample was added to each well. After incubation, a peroxidase conjugate (Protein A) was added. If serum sample tested contains antibodies specific for RHDV, the conjugate will bind to them, whereas if it does not contain specific antibodies no binding will take place. After washing the plate to eliminate all non-fixed material, presence or absence of conjugate was detected by adding a substrate which, in presence of the peroxidase, developed a colorimetric reaction. The assay was then read with a spectrophotometer at 405nm.

Viral RNA Extraction

Total viral RNA was extracted from 140 µl of the tissue supernatants (119) using RNA extraction kit (Qiagen, Germany), according to the manufacturer's protocol and eluted in 60 µl of RNase-free water. The RNAs were used immediately used real-time reverse transcription polymerase chain reaction (RT-PCR) or were kept in -80°C freezer until analysis.

Real-time RT–PCR

By using an indirect ELISA, sera samples obtained from 3832 seemingly healthy rabbits in 25 States spread across Nigeria's six GZs were tested for RHDV antibodies. Naturally occurring antibodies were detected

Five µl of each the RNA extracts were used as template for the real-time RT-PCR. The reactions were performed using the Qiagen QuantiTect® Multiplex RT-PCR kit based on the manufacturer's instruction. Primers and probe which target the VP60 gene which are highly conserved among vast majority of RHDV2 as previously reported by (Duarte *et al.,* 2015) was used in the study at a final concentration of 20 µM for the primers and 20 µM for the probe. The cycling profile used was as follows: RT at 50℃ for 30 min, followed by 40 cycles of initial denaturation at 95℃ for 15 min, denaturation at 95℃ for 30 sec, annealing at 60℃ for 30 sec, elongation at 72℃ for 30 sec and a final extension at 72℃ for 10 min.

Data Analysis

To validate the assay, the ratio OD (Positive Control) / OD (Negative control) must be higher than 10. Cut off Positive/Negative is 0.300. Hence, samples with an OD higher than 0.300 must be considered as positives and samples with OD lower than 0.300 must be considered as negatives. Data obtained from tested sera were analyzed using simple statistical tools such frequency and prevalence.

RESULTS

in 108 rabbits (2.82%), whereas 3,724 rabbits (97.18%) tested negative for RHDV. Seroprevalence based on States, the highest prevalence was found in Ekiti State (11.74%), followed by Ogun State (6.56%),

while the lowest prevalence was found in Akwa Ibom State (0.51%). Zero seroprevalence was recorded in Niger, Taraba, Imo, Edo, Rivers, Nasarawa, Enugu, Benue, Delta, and Plateau States (Table 2). GZs seroprevalence of RHD was as follows: 1.22% in the North Central region, 2.43% in the North East, 2.95% in the North West, 3.09% in the South East, 6.58% in the South West, and 0.66 in the South. South West had the highest seroprevalence, whereas South-South had the lowest seroprevalence (Table 3).

Real-time RT-PCR revealed that all the 119 tissue samples analyzed in this study were negative for RHDV (Table 2).

TABLE I: Showing different geopolitical-zones and states where samples were collected for the study.

Geopolitical Zone	States	No. Sera collected	No. Tissue collected
North-East	Adamawa	75	1
	Bauchi	94	0
	Taraba	158	4
	Yobe	125	66
North-Central	Benue	177	1
	Niger	149	3
	Nasarawa	250	0
	Plateau	272	0
North -West	Kaduna	158	3
	Kano	147	2
South-East	Abia	165	2
	Ebonyi	76	2
	Enugu	92	2
	Imo	82	7
South-West	Ekiti	247	4

	Kwara	218	0
	Lagos	175	1
	Ogun	244	6
	Ondo	199	1
	Oyo	35	7
South-South	Akwa-Ibom	195	1
	Cross-River	145	1
	Delta	67	0
	Edo	243	0
	Rivers	107	2
Total		3832	119

TABLE II: Distribution of rabbit hemorrhagic disease seropositive rabbits based on states in the six geopolitical zones in Nigeria.

Geopolitical Zones	**States**	**No. sera tested**	**No. sera positive (%)**	**No. sera Negative (%)**	**No. tissue tested**	**No. Tissue positive**
North-East	Adamawa	75	1 (1.33)	74 (98.67)	1	0
	Bauchi	94	6 (6.38)	88 (93.62)	0	0
	Taraba	158	0 (0)	158 (100)	4	0
	Yobe	125	4 (3.20)	121 (96.80)	66	0
North-Central	Benue	177	0 (0)	177 (100)	2	0

	Niger	149	0 (0)	149 (100)	3	0
	Nasarawa	250	0 (0)	250 (100)	0	0
	Plateau	272	0 (0)	272 (100)	0	0
North -West	Kaduna	158	3 (1.89)	155 (98.11)	0	0
	Kano	147	6 (4.08)	141 (95.92)	2	0
South-East	Abia	105	8 (7.61)	97(92.39)	5	0
	Ebonyi	76	3(3.95)	73(96.05)	2	0
	Enugu	92	0(0)	92(100)	2	0
	Imo	82	0 (0)	82(100)	7	0
South-West	Ekiti	247	29(11.47)	218(88.53)	4	0
	Kwara	218	13(5.96)	205(94.04)	0	0
	Lagos	172	3 (1.74)	169 (98.26)	1	0
	Ogun	244	16 (6.56)	228(93.44)	6	0
	Ondo	199	10 (5.03)	189(94.97)	1	0
	Oyo	35	1 (2.86)	34 (97.14)	7	0
South-South	Akwa-Ibom	195	1(0.51)	194(99.49)	1	0
	Cross-River	145	4(2.76)	141(97.24)	1	0
	Delta	67	0 (0)	67(100)	0	0
	Edo	243	0 (0)	243(100)	0	0
	Rivers	107	0(0)	107(100)	2	0
TOTAL		**3832**	**108**	**3727**	**119**	**0**

TABLE III: Distribution of rabbit hemorrhagic disease seropositive rabbits based on the geopolitical zones in Nigeria.

Geopolitical Zones	Number Tested	Number Positive	Number Negative	Seroprevalence (%)
North-East	452	11	441	2.43
North-Central	1066	13	1053	1.22
North-West	305	9	296	2.95
South-East	355	11	344	3.09
South-South	757	5	752	0.66
South-West	897	59	838	6.58
Total	**3832**	**108**	**3724**	

DISCUSSION

Although rabbit farming is not as common as poultry and fish farming in Nigeria, rabbitry is recognized as a business that promotes livelihood, hunger relief, and poverty alleviation.

Nigeria's need for rabbit meat has recently increased, leading to a rise in rabbit farming across the country to supply the market. They could be the cause of this recent development due to their high protein content, minimal investment requirements in terms of housing, feeding, and general management, highly prolific nature, and profitability (Daodu *et al*., 2021).

One of the main advantages of employing this laboratory test, aside from the cost-effectiveness and the simplicity of data interpretation, is that we chose ELISA due to the vast quantity of sera samples that can be analyzed and the possibility of detecting

previous exposure to RHDV in the population.

Ekiti State had the highest prevalence in the current study, and there have been multiple reports of outbreaks there. This is a significant finding since Ekiti State shares a border with Kwara State, where the first case of RHDV in Nigeria was documented. The first official report of RHD in Nigeria recorded 74 outbreaks across different states in Nigeria, including Lagos, Oyo, Ekiti, and Kwara States. Importation of rabbit, Lack of financial resources for sustainable surveillance and control have been suggested as major factors in the spread of the virus in Nigeria (Oyewo *et al*., 2022).

Given that more than 90% of the samples tested negative for RHDV and that all of the samples were taken during harmattan (the cold season), we hypothesized that there is only a limited RHDV circulation in rabbits in Nigeria between November and December. The high frequency in the South Western and South Eastern parts of Nigeria may be explained by the region's reputation for receiving a lot of rain. According to Ume *et al*. (2018), rabbits are similarly susceptible to climatic change since their disease resistance deteriorates in damp and drafty environments yet they can survive low temperatures and extreme cold.

Fundamental tools for the diagnosis of viral infections are molecular techniques. While results interpretation is simple, where positivity reflects present or past illnesses. These techniques have a reputation for being sensitive, specific, and employed as techniques for differential diagnoses. Since neither RHDV2 nor RHDV was found during the current RT-PCR analysis, it is reasonable to surmise that the rabbits, who appeared healthy, were maybe infected at some point because antibodies were found. RHDV antibodies were discovered in rabbits of all ages and genders that seemed to be in good condition, raising the possibility that the disease may be present in the rabbit population despite the lack of clinical symptoms. This theory is supported by Garca-Bocanegra *et al*., 2010, that found RHDV in healthy wild rabbits in the absence of significant disease.

CONCLUSION

Our results indicate that the rabbit population in all the Geopolitical Zones of Nigeria were exposed to RHDV. Therefore, it is recommended to develop indigenous vaccines to prevent and control the spread of RHDV in order to alleviate the likely socioeconomic impact of rabbit hemorrhagic disease.

ACKNOWLEDMENTS

This study was supported by the Work Bank Project on Regional Disease Surveillance Enhancement (REDISSE) for sample collection nationwide while reagents for analysis was provided by Freidrich-Loeffler-Institut. Institute for International Animal Health and One Health, Germany. To both organization we are grateful. Our gratitude goes to all surveillance officers involved in sample collection and the Director/ Chief

Executive of the National Veterinary Research Institute, Vom.

REFERENCE

ABRANTES, J. and LOPES, A. M. (2021). A review on the methods used for the detection and diagnosis of rabbit hemorrhagic disease virus (RHDV). *Microorganisms*, *9*(5), 972.

ABRANTES, J., VAN DER LOO, W., LE PENDU, J. and ESTEVES, P. J. (2012). Rabbit haemorrhagic disease (RHD) and rabbit haemorrhagic disease virus (RHDV): a review. *Veterinary research*, *43*(1), 1-19.

ABU, O. A. (1995). Potential of rabbit production as means of supplying animal protein in Nigeria. *Green. Nigeria: Nigerian Association of Agricultural Students, University of Ibadan*, 23-24.

AMBAGALA, A., ABABIO, P., LAMBOO, L., GOOLIA, M., LUNG, O., BERHANE, Y. and ODOOM, T. (2021). Outbreak of rabbit hemorrhagic disease virus 2 infections, Ghana. *Emerging Infectious Diseases*, *27*(7), 1999.

BÁRCENA, J., GUERRA, B., ANGULO, I., GONZÁLEZ, J., VALCÁRCEL, F., MATA, C. P., BLANCO, E. and ALEJO, A. (2015). Comparative analysis of rabbit hemorrhagic disease virus (RHDV) and new RHDV2 virus antigenicity, using specific virus-like particles. *Veterinary research*, *46*, 1-6.

CALVETE, C., MENDOZA, M., SARTO, M. P., BAGÜÉS, M. P. J. D., LUJÁN, L., MOLÍN, J., CALVO, A. J., MONROY F. and CALVO, J. H. (2019). Detection of rabbit hemorrhagic disease virus GI. 2/RHDV2/b in the Mediterranean pine vole (Microtus duodecimcostatus) and white-toothed shrew (Crocidura russula). *Journal of wildlife diseases*, *55*(2), 467-472.

CAPUCCI, L., FUSI, P., LAVAZZA, A., PACCIARINI, M. L. and ROSSI, C. (1996). Detection and preliminary characterization of a new rabbit calicivirus related to rabbit hemorrhagic disease virus but nonpathogenic. *Journal of Virology*, *70*(12), 8614-8623.

DALTON, K. P., ALVARADO, C., REYTOR, E., DEL CARMEN NUÑEZ, M., PODADERA, A., MARTÍNEZ-ALONSO, D., ALONSO, J. M. M., NICIEZA, I., GÓMEZ-SEBASTIÁN, S., DALTON, R. M., PARRA, F. and ESCRIBANO, J. M. (2021). Chimeric VLPs bearing VP60 from two serotypes of rabbit haemorrhagic disease virus are protective against both viruses. *Vaccines*, *9*(9), 1005.

DAODU, O. B., SHAIBU, J. O., RICHARDS, A. B., FOLARANMI, E. B., ADEGOKE, S., AJADI, A., OLORUNSHOLA, I.D., AKANBI, O.B., AFOLABI, A.A., DAODU,

O.C. and AIYEDUN, J.O. and OLUWAYELU, D. O. (2021). Detection and molecular characterization of a first isolate of rabbit haemorrhagic disease virus in Nigeria. *Tropical Animal Health and Production*, *53*, 1-10.

DUARTE, M. D., CARVALHO, C. L., BARROS, S. C., HENRIQUES, A. M., RAMOS, F., FAGULHA, T., LUÍS, T., DUARTE, E. L. and FEVEREIRO, M. (2015). A real time Taqman RT-PCR for the detection of rabbit hemorrhagic disease virus 2 (RHDV2). *Journal of virological methods*, *219*, 90–95. https://doi.org/10.1016/j.jviromet.2015.03.017

ERFAN, A. M. and SHALABY, A. G. (2020). Genotyping of rabbit hemorrhagic disease virus detected in diseased rabbits in Egyptian Provinces by VP60 sequencing. *Veterinary World*, *13*(6), 1098.

FAO (2001). FAO recognizes the increasingly important role of rabbit breeding: Global rabbit production exceeds 1 million tons. Press released 01/57, FAO, Rome, Italy. http://www.fao.org/waicent/ois/press_ne/presseng/2001/pren0157.htm

GARCÍA-BOCANEGRA, I., ASTORGA, R. J., NAPP, S., HUERTA, B., CARBONERO, A., PEREA, A., and ARENAS, A. (2011). Factors affecting the seroprevalence of lagovirus infection in wild rabbits (Oryctolagus cuniculus) in Southern Spain. *The Veterinary Journal*, *189*(1), 89-94.

HÄNSKE, G. G., KÖNIG, P., SCHUHMANN, B., BERTRAM, C. A. and MÜLLER, K. (2021). Death in four RHDV2-vaccinated pet rabbits due to rabbit haemorrhagic disease virus 2 (RHDV2). *Journal of Small Animal Practice*, *62*(8), 700-703.

HU, B., WEI, H., FAN, Z., SONG, Y., CHEN, M., QIU, R., ZHU, W., XU, W., XUE, J. and WANG, F. (2021). Emergence of rabbit haemorrhagic disease virus 2 in China in 2020. *Veterinary Medicine and Science*, *7*(1), 236-239.

INYEINYANG, M. M. and UKPONG, I. G. (2019). The Livestock Sector and its contributions to the Protein and Energy needs of the Nigerian Population. *Ghana Journal of Agricultural Science*, *54*(2), 86-97.

LE PENDU, J., ABRANTES, J., BERTAGNOLI, S., GUITTON, J. S., LE GALL-RECULÉ, G., LOPES, A. M., MARCHANDEAU, S., ALDA, F., ALMEIDA, T., CÉLIO, A. P., BÁRCENA, J., BURMAKINA, G., BLANCO, E., CALVETE, C., CAVADINI, P., COOKE, B., DALTON, K., DELIBES MATEOS, M., DEPTUŁA, W., EDEN, J. S., WANG, F., FERREIRA, C. C., FERREIRA, P., FORONDA, P., GONÇALVES, D., GAVIER-WIDÉN, D., HALL, R., HUKOWSKA-SZEMATOWICZ, B., KERR, P., KOVALISKI, J.,

LAVAZZA, A., MAHAR, J., MALOGOLOVKIN, A., MARQUES, R. M., MARQUES, S., MARTIN-ALONSO, A., MONTERROSO, P., MORENO, S., MUTZE, G., NEIMANIS, A., NIEDŹWIEDZKA-RYSTWEJ, P., PEACOCK, D., PARRA, F., ROCCHI, M., ROUCO, C., RUVOËN-CLOUET, N., SILVA, E., SILVÉRIO, D., STRIVE, T., THOMPSON, G., TOKARZ-DEPTUŁA, B. and ESTEVES, P. (2017). Proposal for a unified classification system and nomenclature of lagoviruses. *Journal of General Virology*, *98*(7), 1658-1666.

LUKEFAHR, S. D. (2007). Strategies for the development of small-and medium-scale rabbit farming in South-East Asia. *Livestock Research for Rural Development*, *19*(9), 34-40.

MAHAR, J. E., HALL, R. N., PEACOCK, D., KOVALISKI, J., PIPER, M., MOURANT, R., HUANG, N., CAMPBELL, S., GU, X., READ, A. and URAKOVA, N. (2018). Rabbit hemorrhagic disease virus 2 (RHDV2; GI. 2) is replacing endemic strains of RHDV in the Australian landscape within 18 months of its arrival. *Journal of virology*, *92*(2), 10-1128.

MAHAR, J. E., READ, A. J., GU, X., URAKOVA, N., MOURANT, R., PIPER, M., HABOURY, S., HOLMES, E.C., STRIVE, T. and HALL, R. N. (2018). Detection and circulation of a novel rabbit hemorrhagic disease virus in Australia. *Emerging Infectious Diseases*, *24*(1), 22.

MCCOLL, K. A., MERCHANT, J. C., HARDY, J., COOKE, B. D., ROBINSON, A. and WESTBURY, H. A. (2002). Evidence for insect transmission of rabbit haemorrhagic disease virus. *Epidemiology & Infection*, *129*(3), 655-663.

MESEKO, C., MILANI, A., INUWA, B., CHINYERE, C., SHITTU, I., AHMED, J., GIUSSANI, E., PALUMBO, E., ZECCHIN, B., BONFANTE, F., MANIERO, S., ANGOT, A., NIANG, M., FUSARO, A., GOBBO, F., TERREGINO, C., OLASOJU, T., MONNE, I. and MUHAMMAD, M. (2023). The Evolution of Highly Pathogenic Avian Influenza A (H5) in Poultry in Nigeria, 2021–2022. *Viruses*, *15*(6), 1387.

OHLINGER, V. F., HAAS, B. E. R. N. D., MEYERS, G. R. E. G. O. R., WEILAND, F. and THIEL, H. J. (1990). Identification and characterization of the virus causing rabbit hemorrhagic disease. *Journal of Virology*, *64*(7), 3331-3336.

ONIFADE, A. A., ABU, O. A., OBIYAN, R. I. and ABANIKANNDA, O. T. F. (1999). Rabbit production in Nigeria: some aspects of current status and promotional strategies. *World rabbit science*, *7*(2), 51-58.

OTU, A., ONWUSAKA, O. C., MESEKO, C., EFFA, E., EBENSO, B., ISONG, I., IJOMANTA, J., OMILEYE, A., EMELIFE, C., EZIECHINA, S., SULEIMAN, K., OCHU, C. and ADETIMIRIN, V. (2024). Learning from One-Health approaches to explore links between farming practices, animal, human and ecosystem health in Nigeria. *Frontiers in Nutrition*, *11*, 1216484.

OYEWO, M., AL-MUSTAPHA, A. I., RICHARDS, B. A., ABDULKAREEM, L., OLASOJU, T., BABALE, S. M. KHALID, H., MESEKO, C. and BALOGUN, M. S. (2022). Preliminary Outbreak Investigation and Spatial Epidemiology of Rabbit Hemorrhagic Disease Outbreak in Nigeria. *Frontiers in Veterinary Science*, *8*, 771599.

QI, R., MIAO, Q., ZHU, J., TANG, J., TANG, A., WANG, X., DONG, D., GUO, H. and LIU, G. (2020). Construction and immunogenicity of novel bivalent virus-like particles bearing VP60 genes of classic RHDV (GI. 1) and RHDV2 (GI. 2). *Veterinary microbiology*, *240*, 108529.

REEMERS, S., PEETERS, L., VAN SCHIJNDEL, J., BRUTON, B., SUTTON, D., VAN DER WAART, L. and VAN DE ZANDE, S. (2020). Novel trivalent vectored vaccine for control of myxomatosis and disease caused by classical and a new genotype of rabbit haemorrhagic disease virus. *Vaccines*, *8*(3), 441.

ROSELL, J. M., DE LA FUENTE, L. F., PARRA, F., DALTON, K. P., BADIOLA SÁIZ, J. I., PÉREZ DE ROZAS, A., BADIOLA DÍEZ, J.J., FERNÁNDEZ DE LUCO, D., CASAL, J., MAJÓ, N., CASAS, J.and FERNÁNDEZ MAGARIÑOS, X. M. (2019). Myxomatosis and rabbit haemorrhagic disease: A 30-year study of the occurrence on commercial farms in Spain. *Animals*, *9*(10), 780.

ROUCO, C., ABRANTES, J., SERRONHA, A., LOPES, A.M., MAIO, E., MAGALHÃES, M.J., BLANCO, E., BARCENA, J., ESTEVES, P.J., SANTOS, N. and ALVES, P.C. (2018). Epidemiology of RHDV 2 (Lagovirus europaeus/GI. 2) in free-living wild European rabbits in Portugal. *Transboundary and Emerging Diseases*, *65*(2), e373-e382.

TUNON, M. J., SANCHEZ-CAMPOS, S., GARCIA-FERRERAS, J., ALVAREZ, M., JORQUERA, F. and GONZALEZ-GALLEGO, J. (2003). Rabbit hemorrhagic viral disease: characterization of a new animal model of fulminant liver failure. *Journal of Laboratory and Clinical Medicine*, *141*(4), 272-278.

UME, S. I., EZEANO, C. I. and ONWUJIARIRI, E. B. (2018). Effect of climate change on rabbit production and choice of adaptation coping strategies by smallholder farmers in

Anambra State, Nigeria. *Int J Innovations Agric Sci*, *2*, 161-173.

WHITE, P. J., TROUT, R. C., MOSS, S. R., DESAI, A., ARMESTO, M., FORRESTER, N. L. GOULD, E.A. and HUDSON, P. J. (2004). Epidemiology of rabbit haemorrhagic disease virus in the United Kingdom: evidence for seasonal transmission by both virulent and avirulent modes of infection. *Epidemiology & Infection*, *132*(3), 555-567.