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Research

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Abstract

Introduction: the rabbit hemorrhagic disease (RHD), is a virulent viral hepatitis. First reported in Nigeria in October 2020, although there had been online record since 2013. Several cases (19,474) and 17,415 mortalities were recorded from 74 outbreaks including Ekiti State. We investigated to verify the existence of the RHD virus and identify possible associated factors. Methods: we conducted an unmatched case-control study among 27 cases and 123 controls. We defined a case as farm with three of the following signs observed in rabbits: brownish diarrhea, epistaxis, coughing, respiratory distress, skin malformation, sudden death and mortality ≥40%, between October 1st to December 13th, 2020. Control defined

as farm that did not meet case definition. We conducted active case search. Multivariate logistic regression was done to identify risk factors of RHDV. **Results:** *the mortality rate was 53%. Twenty-six percent of farms had rabbits´ stock ≤20 and 75% of farmers had training on rabbit farming. Fifty-two percent of the cases were in Ado Ekiti. The following factors were associated with having RHD: having less than 20 rabbits on farm (AOR = 3.98; 95%CI 1.04-15.28) getting water supply from rain (AOR = 22.54; 95%CI 2.26-222.35) sourcing of rabbit stock from Ado-Ekiti (AOR = 25.68; 95%CI 2.63-250.90) disinfecting farm tools (AOR= 0.13; 95%CI 0.03-0.65) and use of commercial feed on farm (AOR= 68.80; 95%CI 2.33-2031).* **Conclusion:** *the outbreak of RHDV in Ekiti State was confirmed clinically, it affected 5 Local Governments Areas. The identified risk factors were, having less than 20 rabbits on farm, using rain water as source of water supply, sourcing of rabbit stock from Ado-Ekiti, not disinfecting farm tools and use of commercial feed on farm. Farmers should source for right breeds in disease free farms and avoid usage of rainwater for the rabbits.*

Introduction

The rabbit hemorrhagic disease (RHD), also known as viral hemorrhagic disease (VHD), is a highly infectious and lethal form of viral hepatitis that affects majorly European rabbits [1]. Morbidity and mortality rates are high in unvaccinated animals. Rabbit hemorrhagic disease virus (RHDV) is thought to have emerged in the 1970s or 1980s in Europe [1,2]. The three strains of RHDV of medical significance are RHDV, RHDVa and RHDV2. Rabbit hemorrhagic disease virus (also referred to as RHDV, RHDV1, or as classical RHD) only affects adult European rabbits (*Oryctolagus cuniculus*), from which it spread to places like Asia, Australia, and elsewhere [3,4]. All viruses causing RHD are extremely contagious. Transmission occurs by direct contact with infected animals, carcasses, bodily fluids (urine, feces, respiratory secretions), and hair. Surviving rabbits may be contagious for

up to 2 months [4]. Contaminated fomites such as clothing, food, cages, bedding, feeders, and water also spread the virus. Flies, fleas, and mosquitoes can carry the virus between rabbits [3,5]. Predators and scavengers can also spread the virus by shedding it in their feces [3]. Caliciviruses are highly resistant in the environment, and can survive freezing for prolonged periods. Importation of rabbit meat may be a major contributor in the spread of the virus to new geographic regions [4]. RHD caused by RHDV and RHDVa demonstrates high morbidity (up to 100%) and mortality (40-100%) in adult European rabbits. Young rabbits 6-8 weeks old are less likely to be infected, and kits younger than 4 weeks old, do not become ill [4,6].

In diagnosing the disease, a tentative diagnosis of RHD can often be made based on clinical presentation, infection pattern within a population, and postmortem lesions. Clinical symptoms vary in different rabbits. In per acute cases, rabbits are usually found dead with no premonitory symptoms [7]. Rabbits may be observed grazing normally immediately before death [3]. In acute cases, rabbits are sedentary and reluctant to move. They may develop a fever up to 42°C (107.6°F) and have increased heart and respiratory rates. Bloody discharge from the nose, mouth, or vulva is common, as is blood in the feces or urine. Lateral recumbence, coma, and convulsions may be observed before death [3]. Rabbits with the acute form generally die within 12 to 36 hours from the onset of fever [7]. Subacute to chronic RHD has a more protracted clinical course, and is more commonly noted with RHDV2 infections. Clinical signs include lethargy, anorexia, weight loss, jaundice, gastrointestinal dilation, cardiac arrhythmias, heart murmurs, and neurologic abnormalities [4]. Death, if it occurs, usually happens 1-2 weeks after the onset of symptoms, and is due to liver failure [7]. Definitive diagnosis requires detection of the virus. As most caliciviruses cannot be grown in cell culture, a variety of molecular tests can be used to identify RHD viruses. Reverse transcription polymerase

chain reaction (RT-PCR) tests are commonly used and considered as accurate testing modality for viruses.

The RHD was first seen in Nigeria in the year 2013, when some farmers imported rabbit breeds from the Republic of Benin, which had earlier outbreaks of the disease that year. It was just in one farm, and Nigeria veterinary public health experts were able to contain it. All rabbits in the farm were immediately destroyed, and the farm was well fumigated [8]. In October 2019 another outbreak of RHD was postulated to have occurred in Nigeria by a newspaper media, when some people again imported infected rabbits from Benin Republic. The outbreak was also contained in the affected farms following destruction of the rabbits and complete fumigation of the farms [2,8]. The first official report of RHD in Nigeria was in June 2020 and as at 27th October 2020, about 19,474 cases had been reported, out of which 17,415 mortalities were recorded from 74 outbreaks across different states in Nigeria including Lagos, Oyo, Ekiti and Kwara States. This study was carried out to identify and line list the positive case farms and commence follow up, to characterize the outbreak in terms of time and place and to determine possible risk factors that may cause further spread of the disease among rabbit farms in Ekiti State.

Methods

Study design: this involved a cross sectional unmatched case-control study, using findings from active case search for RHDV in Ekiti State, with 27 case farms compared with recruited 123 controls, gotten from rabbit farms within Ekiti State that are located close to case farms. Questionnaires were administered to assess risk factors of rabbit farms to RHDV infection.

Study setting: Ekiti State is a southwestern state in Nigeria, the projected population figure for the State stood at 3,655,663 in 2020, with an annual population growth rate of 3.2% [9]. The major

occupation in the State is agriculture, as most of the resources are derived through involvement in personal farming, while there are also a few instances of commercial agricultural ventures. The major agricultural products include yam, cocoa, coffee, plantain, banana, kolanut, palm oil, etc. Many have also started investing in animal protein production through several livestock farming ventures including poultry, piggery, rabbit and cattle rearing [9]. In Ekiti State, there are slightly over 150 rabbit farmers rearing more than 5 thousand rabbits in most of the local government area (LGA) in the state. These farmers are cared for by the state ministry of agriculture, department of veterinary services, who´s facilities are located in the four zones of the state, namely, Ado zone, Ijero zone, Ikole zone and Ikere zone (Figure 1). The state veterinary services in Ekiti are based on this zonal division and there are a total of 16 state veterinary facility, located in each of the LGAs in the state and a standard veterinary laboratory at Ado Ekiti serves all the facilities.

Epidemiological investigation

Study population: the study population includes all rabbit farms in Ekiti State.

Inclusion criteria: all rabbit farms present in the farm registration records of the surveillance unit of the department of veterinary services in Ekiti State, between 30th November to 13th December 2020.

Exclusion criteria: all rabbit farms in the inclusion criteria that does not grant permission to be interviewed.

Sample determination: a complete sampling of all rabbit farms present in the records of the surveillance unit of the department of veterinary services, Ekiti State was adopted for this study.

Case definition: cases were defined using a case definition adopted from that published by the federal department of veterinary and pest control service, Nigeria. Confirmed case: any farm with

rabbits that are laboratory confirmed of RHDV or farm in which three of the following signs are observed in their rabbits: brownish diarrhea, epistasis, violent shaking of the rabbits/incoordination, coughing, respiratory distress, skin malformation/coloration, sudden death and mortality up to 40% or above in the affected LGAs in Ekiti State between 1st October to 13th December 2020.

Control: the farms selected for controls were rabbit farms located within Ekiti State during the period of our study, that did not meet the case definition of RHD as indicated for case farms but could be exposed to the same RHD risk factors as case farms.

Active case search: we conducted active case search and assessment of rabbit farmer´s practices of biosecurity and IPC on their farms. Efforts were made to find a possible source (primary case) and other risk factors that can contribute to the propagation of the disease statewide. At the facilities visited, farm records were checked and farm owner (worker) interviewed. A total of 150 farms (complete sampling) were visited and served with the same questionnaire by an interviewer.

Generating hypothesis: five hypotheses were generated from findings of the active case search, and tested with data from the questionnaire distributed to farmers: having no training on rabbit production was contributory to being in case farm; having below 20 rabbits in farm has a relationship with being in case farm group; not keeping good IPC management practices is associated to having cases of RHD; purchasing stocking rabbit in Ado Ekiti is related to being in the case farm group; source of water supply to farm is related to being in the case farm group.

Testing hypothesis: this was done by comparing responses of respondent from case farms with those of control farms for various aspect of farm management RHD risk factor variables.

Data source: data for all variables to be measured was extracted from Kobotool where the responses for the questionnaire were collected and stored. A semi-structured questionnaire was used for this study. The questionnaire was divided into 4 parts where all data for the variables of interest were derived. The parts included the demographic characteristics of farms, the farm management practices, the IPC practices on farm and the clinical history of the farm.

Data analysis: data cleaning, tables and graphs were prepared using Microsoft Excel 2016. Charts were used to present epidemiologic distributions in terms of person, place, and time. QGIS 2.18 was used to extract the data features, like most affected LGA and the zonal distribution of affected cases for the period under review. The epi-curve based on date of rabbit blood sample collection was also generated. A chi-square analyses was done using Epi_info_7. We calculated odds ratios with 95% confidence limit, and those that attained significance, to rule out possible bias, were subjected to logistic regression for multivariate analysis, significance at this level was determined at $P<0.05$.

Results

A total of 27 (18%) farms interviewed were classified as cases between 30th November and 13th December 2020 based on the exhibition of symptoms by their rabbit, that is in conformity with the case definition of RHD. Of the case farms, 25 (92.6%) had owners <60 years of age and owners with only secondary school education had the highest relative proportion of (87.5%) in the case farm having less than 20 rabbits in farm, having majorly black giant breeds of rabbit in farm, sourcing rabbits from Ado-Ekiti, using rain water to supply drinkers, using self-made or commercial made feeds on farm, not washing hands when handling rabbit, not disinfecting farm tools after use, presence of cull pen for sick rabbits and fencing of farms. The predictors of RHD in farms in Ekiti State includes, sourcing rabbits from Ado-

Ekiti, having stock size less than 20 rabbits on farm, using rain water as source of drinking water to rabbit, not disinfecting farm tools and feeding rabbit with commercially produced feed (Table 3). Number of case farms of RHD was highest in Ado-Ekiti LGA and reduces as we move away from the capital city (Figure 2). A time trend of date of sampling of RHD positive farms showing a propagated type of chart (Figure 3).

Discussion

During the period of the RHD outbreak investigation in Ekiti State, 27 (18%) of the farms interviewed fell into the case farm group, likewise factors such as, the source of rabbit stock for the farm, having less than 20 rabbit stock size on farm, using rain water as source of water supply to farm rabbits, not disinfecting farm tools after use and using commercially made rabbit feed as source of rabbit food, were predictors of RHD infection in rabbit farms. This is useful knowledge among farmers and veterinary practitioners to contain further spread of the disease among rabbit farms in Ekiti State. There were more male rabbit farmers in 81.3% in Ekiti State as at December 2020. This is in contrast to a 2012 publication of a higher female rabbit farmer population in the state [10,11]. A higher number of 77.3% of people younger than the retirement age of 60 was observed in our study, different from the dominance of post retirement rabbit farmers noticed in Ekiti in the 2012 study. This development can be linked to the ongoing state government agricultural initiative that involves more young farmers in the state. However, farmers with below 20 rabbits in their farms still remain the largest population of rabbit farmers, even though more farmers are now increasing their stock, which is evidence from the reduction in the below 20 rabbits farmer´s population from 73.1% of rabbit farmers in 2018 [12] to 50.7% in our present study.

In Ekiti State, the majority of the rabbit farming activity is resident in Ado-Ekiti LGA [12] and

according to our study, 51.9% of the farms with cases of RHD are in Ado-Ekiti. Most other LGA in the state acquire their source rabbits and feeds from Ado-Ekiti, hence disease transmission tends to emanate from the capital and spread to other LGA´s in Ekiti State as seen in Figure 3 above. LGAs like Irepodun/Ifelodun that borders Ado-Ekiti has a higher relative incidence of case farms compared to other much farther LGA´s in the state. Occurrence of case farms were not in accordance to the four zonal veterinary farm outreach division of the state, hence ruling out the spread of RHD fomites through routine inspections or interventions carried out on rabbit farms by state veterinarian officers. The time trend, shows an increasing trend of cases. All cases of RHD were also mortalities with a zero survival rate. The mortality rate of RHD has been published to be between 30-100% [13] but the poor survival rate among rabbits in Ekiti State may be related to stress experienced by rabbits imported to a new environment, due to availability of few indigenous breeds in Nigeria commercial agriculture. The epidemic curve for RHD is ideal for a propagated outbreak, with several peaks recorded at various intervals on the curve [12].

Generated hypothesis from the descriptive epidemiology conducted, were tested, and this gave insight on the likely predictors responsible for the propagations and spread of the RHD virus in the state. Farmers with lower than 20 rabbit on their farms were about four times as likely to have cases of RHD than those with greater than 20 rabbits on their prevention and control (IPC) practices than larger scale farmer, hence disease transmission will be easier in the small-scale farms. The source or location where the rabbit stock was acquired and source of water supply also contributed significantly to the possibility of a farm having cases of RHD. Farms that obtained their water majorly from rainwater had 22 times the odd of having RHD in their farms, this is likely because farmers get this rain water by putting buckets underwater coming down from rooftops where rats, lizards, bird and wild rabbit must have

dropped dungs. Also, because these buckets are not covered, they serve as a source for breeding mosquitoes and other flies that help the spread of RHD [3,14]. Despite our findings, we were however limited in our ability to carry out a laboratory confirmation of the cases of RHD detected in our study, but the importance of an information about this disease in Nigeria totally justifies our using clinical diagnosis as a link to the earlier confirmed RHD cases in the country. Further study is therefore recommended in the future, to further buttress our findings when there is readily available laboratory diagnosis.

Conclusion

The outbreak of RHDV in Ekiti State was confirmed clinically, it affected 5 LGAs. The identified risk factors were, stock size of the farm, source of water supply, source of rabbit stock, not disinfecting farm tools and use of commercial feed on farm. We recommended that farmers should source for right breeds in disease free farms and avoid usage of rainwater for the rabbits. We advocated technical support to institutionalize IPC practices at rabbit farms also for continuous farmer monitoring.

What is known about this topic

- *RHD has been known as a viral hemorrhagic disease that affects rabbit of all ages;*
- *It is more virulent in rabbits of European origin, and the virus is spread through direct contact of susceptible rabbit with infected ones;*
- *Mechanical spread is possible through flies and mosquitoes.*

What this study adds

- *There is a clinical confirmation of rabbit hemorrhagic disease in Ekiti State Nigeria;*

- *The identified risk factors of RHD in Ekiti State Nigeria are: having less than 20 rabbits on farm, using rain water as source of water supply, sourcing of rabbit stock from Ado-Ekiti, not disinfecting farm tools and use of commercial feed on farm.*

Competing interests

The authors declare no competing interests.

Authors' contributions

FOS drafted the manuscript, collected the data and also performed the statistical analysis. AU, MSB and TO revised the manuscript critically for important intellectual content, MSB drafted the abstract. All the authors listed read and approved the final manuscript.

Acknowledgments

Tables and figures

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Figure 1: map of Nigeria, showing zonal division of Ekiti State

Figure 2: Ekiti RHD cases by farm and LGA, 30th November to 13th December 2020

Figure 3: Ekiti RHD case farms by date of sampling, 1st to 11th December 2020

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Table 1: management practice and demographic distribution among case and control farms		
Variables	Cases (%) n=27	Control (%) n=123
Location of farm (LGA)		
Ado	14 (51.9)	99 (80.5)
Ikere	3 (11.1)	4 (3.3)
Irepodun/Ifelodun	7 (25.9)	8 (6.5)
Oye	3 (11.1)	12 (9.7)
Number of rabbits in farms		
≤ 20	20 (74.1)	56
21-50	3 (11.1)	35
51-70	3 (11.1)	21
Above 70	1 (3.7)	11
Type of training before rabbit production		
None	1 (3.7)	4 (3.3)
Informal	20 (74.1)	106 (86.2)
Formal	6 (22.2)	13 (10.5)
Stock from Ado-ekiti		
Yes	9 (33.3)	15 (12.2)
No	18 (66.7)	108 (87.8)
Feed used on farm		
Self-made feed Yes	19 (70.4)	111 (90.2)
No	8 (29.6)	12 (9.8)
Kitchen waste Yes	7 (25.9)	16 (13.0)
No	20 (74.1)	107 (87.0)
Vegetable alone Yes	13 (48.1)	67 (54.5)
No	14 (51.9)	56 (45.5)
Commercial feeds Yes	25 (92.6)	83 (67.5)
No	2 (7.4)	40 (32.5)
Keeping farm records. Yes	16 (59.3)	49 (39.8)
No	11 (40.7)	74 (60.2)
Washing hands when handling rabbit. Yes	12 (44.4)	88 (71.5)
No	15 (55.6)	35 (28.5)
Use designated cloth on farm. Yes	5 (18.5)	26 (21.1)
No	22 (81.5)	97 (78.9)
Disinfect farm tools Yes	4 (14.8)	53 (43.1)
No	23 (85.2)	70 (56.9)
Presence of cull pen for sick rabbit. Yes	21 (77.8)	68 (55.3)
No	6 (22.2)	55 (44.7)
Farm restricted by fencing. Yes	22 (81.5)	71 (57.8)
No	5 (18.5)	52 (42.2)
Contact with other farm animals		
Yes	11 (40.7)	44 (35.8)
No	16 (59.3)	79 (64.2)
Water supplied to rabbit		
Well water		
Yes	15 (55.6)	68 (55.3)
No	12 (44.4)	55 (44.7)
Rain water		
Yes	7 (25.9)	2 (1.6)
No	20 (74.1)	121 (98.4)
Stream water		
Yes	3 (11.1)	4 (3.3)
No	24 (88.9)	119 (96.7)
Tap water		
Yes	2 (7.4)	8 (6.5)
No	25 (92.6)	115 (93.5)
Bore-hole		
Yes	11 (40.7)	57 (46.3)
No	16 (59.3)	66 (53.7)

Table 2: management factors associated with RHD infection in rabbit farms, 30th November to 13th December 2020				
Variable	CASE (%) n=27	CONTROL (%) n=123	OR 95% Confidence interval	P-value
Training on rabbit farming				
Not trained	1 (20.0)	4 (80.0)	1.14 (0.12-10.66)	0.63
Trained	26 (17.9)	119 (82.1)		
Number of rabbit in farm				
≤ 20 rabbits in farm	20 (26.3)	56 (73.7)	3.39 (1.35-8.67)	0.006*
Above 20 rabbits in farm	7 (9.5)	67 (90.5)		
Breed of rabbit in farm				
Chinchilla breed				
Yes	11 (14.1)	67 (85.9)	0.58 (0.25-1.34)	0.21
No	16 (22.2)	56 (77.8)		
Harlequin breed				
Yes	4 (36.4)	7 (63.6)	2.88 (0.78-10.65)	0.11
No	23 (16.5)	116 (83.5)		
New Zealand breed				
Yes	14 (25.9)	40 (74.1)	2.23 (0.87-5.20)	0.06
No	13 (13.5)	83 (86.5)		
Black giants breed				
Yes	6 (54.5)	5 (45.5)	6.74 (1.89-22.1)	0.005*
No	21 (15.1)	118 (84.9)		
Stock from Ado-ekiti				
Yes	9 (37.5)	15 (62.5)	3.6 (1.37-9.45)	0.02*
No	18 (14.3)	108 (85.7)		
Source of water to farm				
Well water				
Yes	15 (18.1)	68 (81.9)	1.01 (0.44-2.34)	1.00
No	12 (17.9)	55 (82.1)		
Rain Water				
Yes	7 (77.8)	2 (22.2)	21.17 (4.10-109.29)	0.00008*
No	20 (14.2)	121 (85.8)		
Bore hole				
Yes	11 (16.2)	57 (83.8)	0.80 (0.34-1.85)	0.67
No	16 (19.5)	66 (80.5)		
Stream water				
Yes	3 (50.0)	3 (50.0)	5.00 (0.95-26.28)	0.07
No	24 (16.7)	120 (83.3)		
Feed used on farm				
Self-made feed Yes	19 (14.6)	111 (85.4)	0.26 (0.09-0.71)	0.01*
No	8 (40.0)	12 (60.0)		
Kitchen waste Yes	7 (30.4)	16 (69.6)	2.34 (0.85-6.81)	0.14
No	20 (15.7)	107 (84.3)		
Commercial feeds Yes	25 (23.1)	83 (76.9)	6.02 (1.36-26.70)	<0.01*
No	2 (4.8)	40 (95.2)		
Washing hands when handling rabbit. Yes	12 (12.0)	88 (88.0)	0.32 (0.14-0.75)	0.01*
No	15 (30.0)	35 (70.0)		
Use designated cloth on farm. Yes	5 (16.1)	26 (83.9)	0.85 (0.29-2.46)	1.00
No	22 (18.5)	97 (81.5)		
Disinfect farm tools Yes	4 (7.0)	53 (93.0)	0.23 (0.08-0.70)	<0.01*
No	23 (24.7)	70 (75.3)		
Presence of cull pen. Yes	21 (23.6)	68 (76.4)	2.83 (1.07-7.50)	0.03*
No	6 (9.8)	55 (90.2)		
Farm fenced. Yes	22 (23.7)	71 (76.3)	3.22 (1.15-9.07)	0.03*
No	5 (8.8)	52 (91.2)		

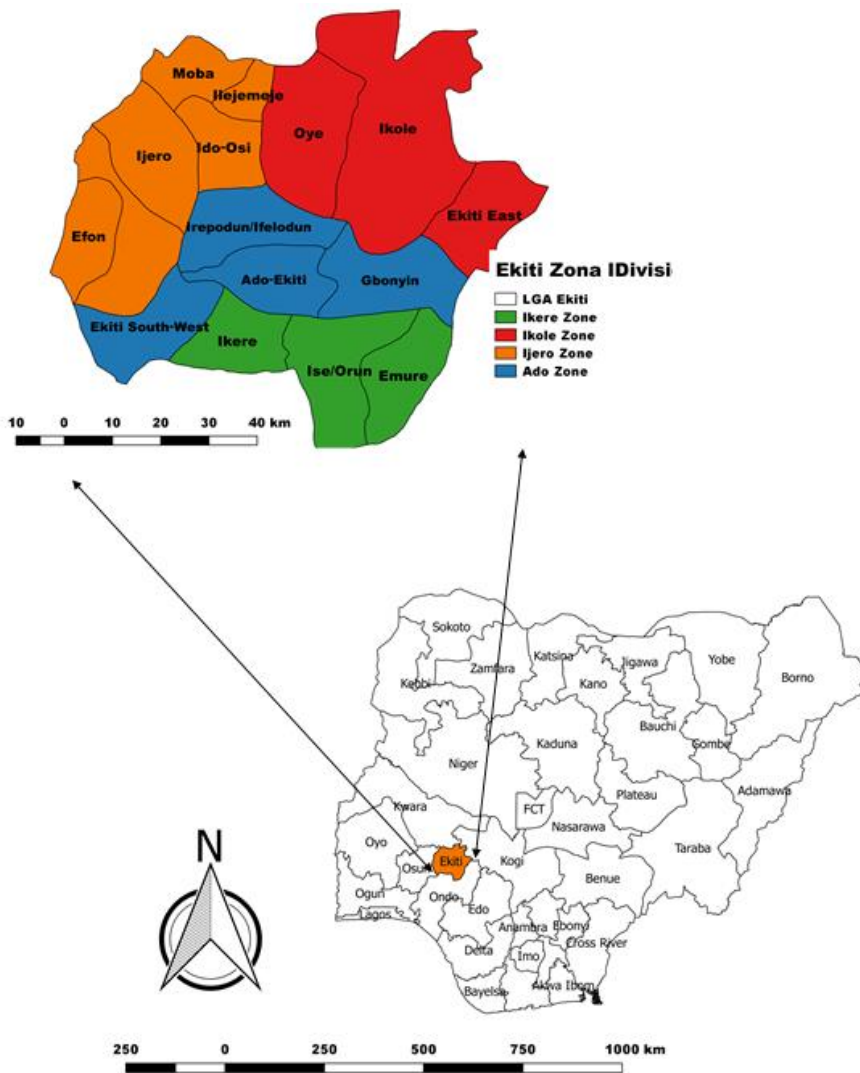| Table 3: predictors of RHD infection in rabbit farms in Ekiti State, 30th November to 13th December 2020			
Variables	**Adjusted Odds Ratio**	**95% Confidence Interval**	**P-value**
Are major farm breeds black giants breed? Yes/No	1.09	(0.14-8.64)	0.94
Rabbits sourced from Ado? Yes/No	25.68	(2.63-250.90)	<0.01*
Number of rabbits on farm ≤20 / >20	3.98	(1.04-15.28)	0.04*
Rain water used on farm Yes/No	22.54	(2.26-224.35)	<0.01*
Disinfect farm tools Yes/No	0.13	(0.03-0.65)	0.01*
Farm restricted by fencing Yes/No	3.91	(0.91-16.85)	0.07
Presence of cull pen for sick rabbit Yes/ No	3.43	(0.82-14.28)	0.09
Rabbit fed on commercial feed Yes/No	68.80	(2.33-2031)	0.01*
Rabbit fed from self-made feed Yes/No	6.53	(0.23-183.84)	0.27
Washing hands before handling rabbit Yes/No	5.29	(0.90-30.94	0.07

Figure 1: map of Nigeria, showing zonal division of Ekiti State
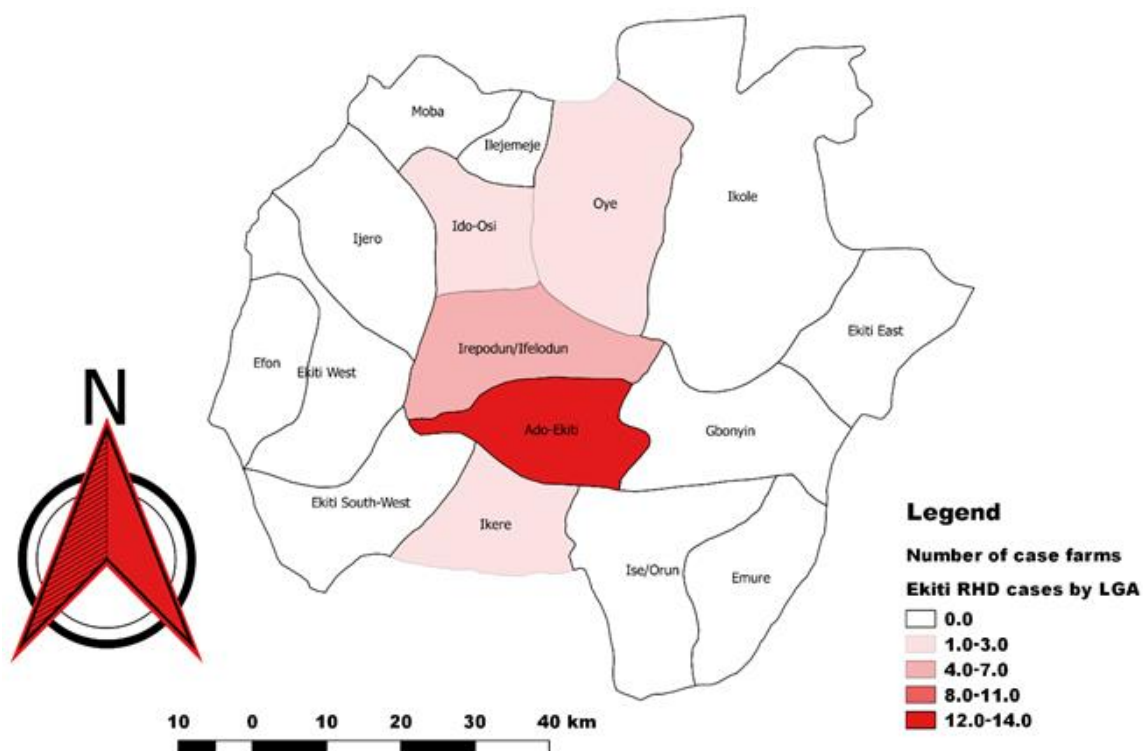
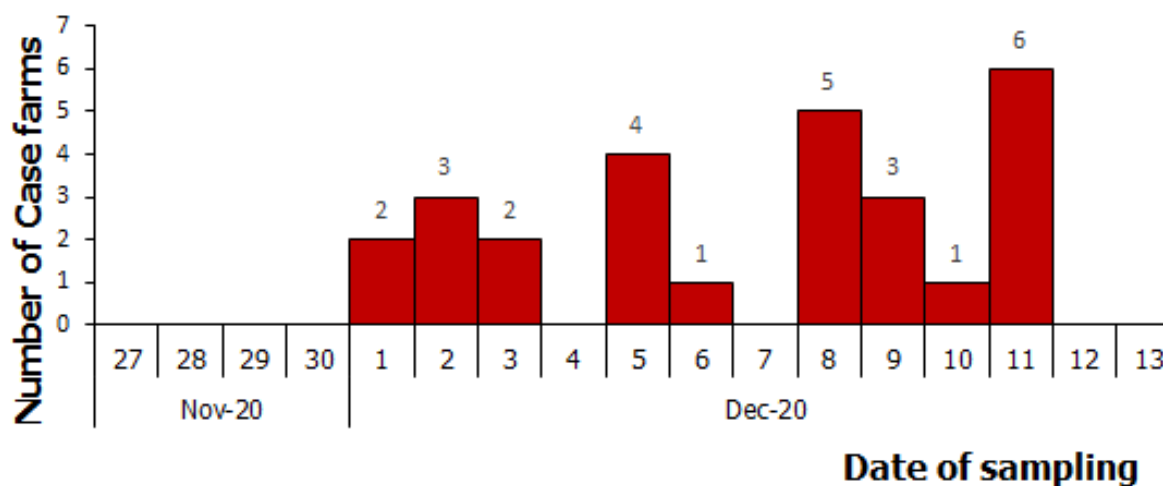**Figure 2**: Ekiti RHD cases by farm and LGA, 30th November to 13th December 2020

Figure 3: Ekiti RHD case farms by date of sampling, 1st to 11th December 2020