

Research article**Biosecurity practices and occurrence of rabbit hemorrhagic disease in southern Benin**Mensah SEP¹, Atchadé GST¹, Montcho M², Adjahoutonon KYKB^{1*}, Lahamy O¹, Koudandé OD¹, Mensah GA¹

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<p>Article history Received: 11 Sep, 2019 Revised: 20 Oct, 2019 Accepted: 11 Nov, 2019</p>	<p>Abstract</p> <p>Rabbit production in Benin has been seriously affected by many outbreaks of Rabbit Haemorrhagic Disease between 1995 and 2015 which continue to be a major constraint for this activity. A cross sectional survey was done from October to November 2018 in Research and Development Sites of southern Benin to carry out the typology of rabbit farms considering biosecurity practices and vaccination against RHD and determine the influence of each type of rabbit farms on RHD occurrence. Forty rabbit farms were randomly sampled in height sites located respectively in municipalities of Kétou, Adjohoun, Sèmè-Podji, Tori-Bossito, Grand-Popo, Djakotomè, Zogbodomey and Sakété. Blood samples were randomly collected by ear vein from one buck, two does and two growing rabbits from each of the sampled farms for antibodies against both RHDV and RHDV2 detection by competitive ELISA. Factor Analysis of Mixed Data and Hierarchical Clustering on Principal Components were performed for farm typology and quasipoisson model was run to check the influence of farm types on RHD occurrence. Surveyed rabbit farmers were mostly men (94.74%), are 45±13 years old in average, had secondary school (36.84%) or university (36.84%) level. They were very often monogamous (68.42%) and owned 111±118 rabbits in average. Three groups of similar farms had been identified: Group 1 and 3 characterized by disinfection of building and materials and use of footbaths with effective disinfectants, droppings collection, buildings cleaning and respectively not polygamous households and modern buildings; group 2 where farmers did not disinfect cages, buildings or feeders and waterers and did not use footbath, had polygamous household and did not have high education level. All the rabbits in surveyed farms were free from antibodies against the two variants RHDV1 and RHDV2 of RHD at the time of the study. Otherwise, RHD cases since 2015 were lower in farms in group 2 than in farms in groups 1 and 3. As biosecurity practices were better in groups 1 and 3 than in group 2, it may be inferred that the farms which have more previous cases of RHD observe more biosecurity measures.</p> <p>Keywords: RHD, rabbit farms, typology, southern Benin</p>
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Introduction

Small livestock species, including rabbits, have been promoted as tools for poverty alleviation programs (Owen et al., 2005). For over 3 decades now, the contribution of smallholder rabbit units to food security in developing countries has been clearly recognized (Chrysostome et al., 2011). The support of global organizations and foundations for rabbit research and development has been ongoing for several decades now. Of special interest is the support provided by the Food and Agriculture Organization (FAO) of the United Nations for rabbit projects in several developing countries (Belli et al., 2008). Rabbit is particularly favored for poverty reduction programs on account of its low investment and early benefits, and subsistence on renewable resources for feeding, housing and general management. Thus, small-scale rabbit projects could be used to fight poverty. Several reports have established favorable impacts of rabbit development projects in terms of: (a) poverty alleviation (Lukefahr, 2007; Owen et al., 2005); (b) rural development (Kpodekon et al., 2000); (c) entrepreneurial skills (Kaplan-Pasternak, 2011); (d) humanitarian services including recovery efforts from natural disasters (Kaplan-Pasternak and Lukefahr, 2011). However, Rabbit farming in Benin is constrained by occurrence of Rabbit Hemorrhagic Disease (RHD), a highly contagious and fatal disease (OIE, 2018b). After several outbreaks from 1995 to 2015, this disease is now endemic in the country and causes important economic losses in rabbit farms (Kpodekon et al., 2000). The high incidence of infectious disease in rabbit farms raises questions with respect to on-farm disease prevention and management practices. Factors that may contribute to the persistence of infectious diseases include intensification of rabbit farming, determinants of rabbit farming systems and farm typology (Brash et al., 2017).

This study aims to carry out the typology of rabbit farms considering farms characteristics, biosecurity practices and vaccination against RHD in order to understand the influence of each type of rabbit farms on RHD occurrence.

Materials and Methods

Study area

The study was carried out in eight municipalities in southern Benin: Kétou, Adjohoun, Sèmè-Podji, Tori-Bossito, Grand-Popo, Djakotomè, Zogbodomey and Sakété (Fig. 1). In each municipality, one village previously selected by the National Institute of Agricultural Researches of Benin as Research and Development Unit was chosen, respectively Adapklamè, Houinsa, Kpodji-Agué, Hayakpa, Kpovidji, Zouzouvou,

Tovlamè, and Sahoro-Nagot. The average annual rainfall in this area varies from 1100 and 1200 mm with a dry season of up to five months. The average annual temperature ranges from 24.9 to 31.1 °C according to data collected between 2008 and 2010 (INSAE/Bénin, 2012). The vegetation is dominated by forest, woodland and savannahs on different soil types: ferruginous, ferralitic and hydromorphic soils (Akoègninou *et al.*, 2006). The proportion of the active population involved in agriculture in these municipalities varies from 4.3 to 65.9% (INSAE/MDAEP/Benin, 2016).

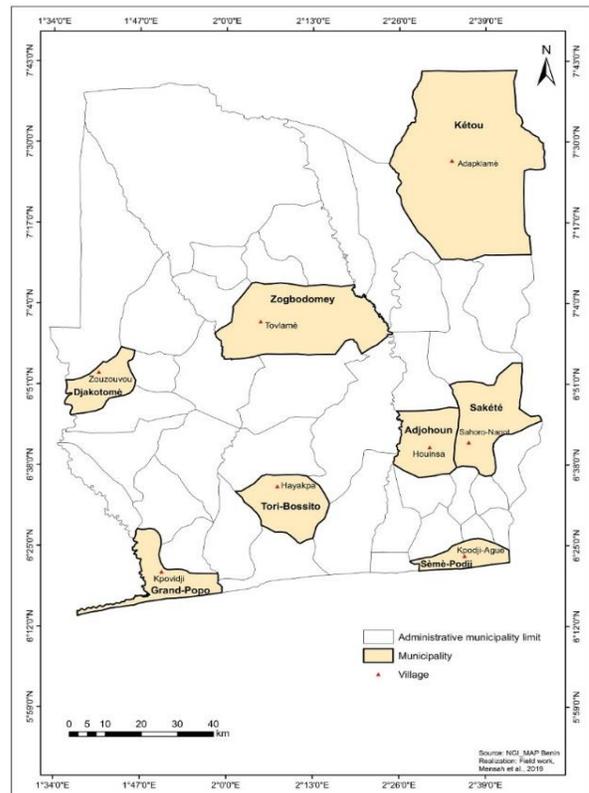


Fig. 1: Study area

Data collection

A cross-sectional survey was done in the rabbit farms in these eight villages from October to November 2018. Data on (i) farms characteristics, (ii) livestock characteristics, (iii) health and biosecurity, (iv) vaccination against RHD and (v) RHD occurrence were collected using a semi-structured questionnaire through face-to-face interviews. Five rabbit farms were randomly chosen and surveyed per village, making forty farms for the study area. In each of the selected farms, blood samples were randomly collected by ear vein from one buck, two does and two growers for antibodies against both RHDV and RHDV2 variants of RHD detection by competitive ELISA (OIE, 2018a).

Statistical analysis

Farms with missing data, variables with less than 5% of observations and variables with one modality were removed from the database. Factor Analysis of Mixed Data (FAMD) was performed on the variables related to farms characteristics, biosecurity and vaccination against VHD. Hierarchical Clustering on Principal Components (HCPC) was performed on the coordinates of the respondents in the factor map formed by the first two dimensions of the FAMD to identify groups of similar farms. Finally, quasipoisson model was used to access the influence of these groups of similar rabbit farms on the numbers of RHD cases occurred in the sampled farms from 2015 to 2018.

All the analyses were performed in Microsoft Excel 2013 and R 3.5.1 with the packages 'FactoMine ytfRR', 'factoextra' and 'Ggplot2'.

Results

Socio-demographic profile of rabbit farmers

The surveyed rabbit farmers were mostly men (94.74%) with an average age of 45 ± 13 years. Most of them reached secondary school (36.84%) or university (36.84%). Few reached primary school (18.42%) and only 7.89% are not educated but literate. They were very often monogamous (68.42%) or polygamous (28.95%) and very few are bachelor (2.63%). The number of rabbits owned by them varies from 6 to 616 with an average of 111 ± 118 rabbits.

Typology of rabbit farms considering biosecurity and vaccination practices

The first two dimensions in the FAMD explained 17.66% of the total variance. The variables which mostly contribute to the first dimension are footbath filling frequency, use of footbath, disinfectant for footbath, number of previous RHD cases, type of building, disinfectant for cage, cage disinfection

frequency, feeders and waterers disinfection frequency, village, disinfectant for building and disinfectant for feeders and waterers.

The most important variables in dimension 2 are building cleaning frequency, droppings collection frequency, disinfectant for feeders and waterers, cage cleaning frequency, feeders and waterers disinfection frequency, building disinfection frequency, disinfectant for building, disinfectant for cage, village, cage disinfection frequency, type of household and education level.

The Hierarchical Clustering on Principal Components identified three clusters or groups of similar rabbit's farms from the coordinates on the first two dimensions (Fig. 2). The most critical variables in the constitution of these groups are showed in table 1.

Group 1 was characterized by the use of sodium hypochlorite as disinfectant for feeders and waterers, cages and building by most of the farmers. Droppings was collected and buildings were cleaned every week. Buildings were disinfected every month and cages cleaned every 2 months. Households were mainly not polygamous.

In group 2, most of farmers did not disinfect cages, buildings or feeders and waterers and did not use footbath. The ones who use footbath did not fill it with commercial disinfectant mixed formulas containing aldehydes, ammoniums and alcohols or phenol. Sodium hypochlorite was used by few of them as disinfectant for buildings but not for cage or feeders and waterers. Paradoxically, they clean building every day. They are polygamous in majority and did not have university education level.

In group 3, footbaths were frequently used and filled every day with commercial disinfectant mixed formulas containing aldehydes, ammoniums and alcohols or with phenols. Cage were mainly disinfected every month with phenol and buildings were modern and not semi-modern.

Table 1: Critical variables in the construction of groups of similar rabbit's farms

Variables	Df	p value
Use of footbath	2	<0,000***
Disinfectant for footbath = None	2	<0,000***
Disinfectant for footbath = Mixed Aldehyde, Ammonium, Alcohol	2	<0,000***
Footbath filling frequency	6	<0,000***
Disinfectant for feeders and waterers = Hypochlorite	2	<0,000***
Disinfectant for footbath = Phenol	2	<0,000***
Disinfectant for cage = Hypochlorite	2	<0,000***
Disinfectant for building = Hypochlorite	2	<0,000***
Disinfectant for cage = Phenol	2	0,004**
Disinfectant for feeders and waterers = None	2	0,005*
Village	14	0,005*
Building cleaning frequency	8	0,016*
Type of building	6	0,024*
Droppings collection frequency	8	0,026*

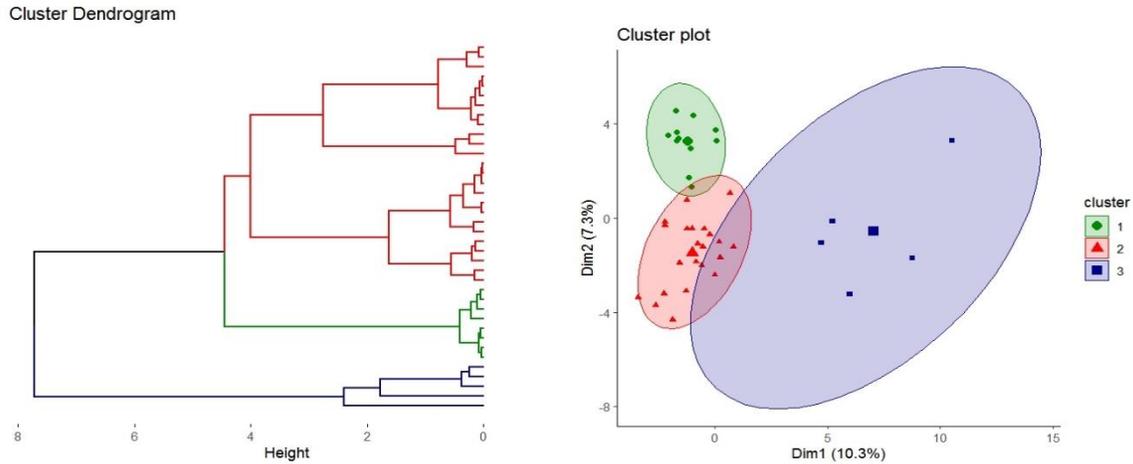


Fig. 2: Cluster dendrogram and plot for identification of groups of similar rabbit farms. The characteristics of three identified farmers groups are showed in tables 2. The groups 1, 2, and 3 contain respectively 11, 22 and 5 farms.

Table 2: Characteristics of rabbit farmers in the three groups

Variables	Modalities	Group 1 (n=11)				Group 2 (n=22)				Group 3 (n=5)			
		Class/ Mod/	Class/ Mod/	p	V	Class/ Mod/	Class/ Mod/	p	V	Class/ Mod/	Class/ Mod/	p	V
Disinfectant for feeders and waterers Hypochlorite	Yes	100.00	72.73	<0,000	4.65	0.00	0.00	<0,000	3.65				
	No	10.00	27.27	<0,000	-4.65	73.33	100.00	<0,000	3.64				
Disinfectant for feeders and waterers None	Yes	5.26	9.09	0,002	-3.16	78.95	68.18	0.011	2.53				
	No	52.63	90.91	0,002	3.16	36.84	31.8	0.011	-2.53				
Disinfectant for cage Hypochlorite	Yes	100.00	54.55	<0,000	3.76	0.00	0.00	0.003	-2.98				
	No	15.62	45.45	<0,000	-3.76	68.75	100.00	0.003	2.98				
Disinfectant for cage Phenol	Yes									60.00	60.00	0,011	2.54
	No									6.06	40.00	0,011	-2.54
	Yes					76.47	59.09	0.045	2.00				
Disinfectant for cage None	No				42.86	40.91	0.04	-2.00					
Disinfectant for building Hypochlorite	Yes	87.50	63.64	<0,000	3.73	12.50	4.54	0.006	-2.77				
	No	13.33	36.36	<0,000	-3.73	70.00	95.45	0.006	2.77				
Disinfectant for building Unspecified	Yes					100.00	27.27	0.027	2.21				
	No					50.00	72.73	0.027	-2.21				
Use of footbath	Yes					0.00	0.00	0.009	-2.62	100.00	100.00	<0,000	4.75
	No					66.67	100.00	0.009	2.62	0.00	0.00	<0,000	-4.75
	Yes									100.00	60.00	0,001	3.24
Disinfectant for footbath Phenol	No									5.71	40.00	0,001	-3.24
Disinfectant for footbath Mixed Aldehyde Ammonium Alcohol	Yes					0.00	0.00	0.025	-2.25	100.00	80.00	<0,000	3.98
	No					64.7	100.00	0.025	2.25	2.94	20.00	<0,000	-3.98
Disinfectant for footbath None	Yes					66.67	100.00	0.009	2.62	0.00	0.00	<0,000	-4.75
	No					0.00	0.00	0.009	-2.62	100.00	100.00	<0,000	4.75
Cage disinfection frequency	1 month									42.86	60.00	0.037	2.09
Building disinfection frequency	1 month	54.54	54.54	0,041	2.04	27.27	13.64	0.021	-2.30				
	Never	0.00	0.00	0,018	-2.37	90.00	40.91	0.020	2.33				
Feeders and waterers disinfection frequency	1 week					33.33	18.18	0.049	-1.97				
	Never					66.67	100.00	0.009	2.62	0.00	0.00	<0,000	-4.75
Footbath filling frequency	1 day									100.00	60.00	0,001	3.24
	1 week	69.23	81.82	<0,000	3.73	23.08	13.64	0.003	-3.00				
Droppings collection frequency	1 day	10.53	18.18	0.016	-2.40								
	2 months	100.00	27.27	0,020	2.33								
Cage cleaning frequency	1 day					80.00	54.54	0.032	2.15				
	1 week	69.23	81.82	<0,000	3.31	23.08	13.64	0.003	-3.00				
Building cleaning frequency	1 day	5.00	9.09	<0,000	-3.36	80.00	72.73	0.005	2.81				
Village	Kpovidji	100.00	45.45	<0,000	3.31	0.00	0.00	0.009	-2.62				
	Modern									60.00	60.00	0,011	2.54
Type of building	Semi-modern									3.45	20.00	0,008	-2.66
	Polygamous	0.00	0.00	0,011	-2.54	90.91	45.45	0.010	2.58				
Type of household	Monogamous					46.15	54.54	0.037	-2.08				

Table 3: Parameters in the quasipoisson model

	Estimate	e ^(Estimate)	Std. Error	p value
Intercept	3.47	32.14	0.67	<0.000***
Group2	-0.95	0.39	1.01	0.356
Group3	1.68	5.37	0.79	0.041*

Moreover, no antibodies against RHDV nor RHDV2 has been detected in rabbit's sera sampled from study area. In the quasipoisson model (table 3), RHD cases during the past three years were 0.39 times lower for the farms in group 2 than the farms in group 1, but this difference is no significant ($p>0.05$). Contrariwise, the farms in group 3 had significantly 5.37 times more RHD cases from 2015 to 2018 than the farms in group 1 ($p<0.05$).

Discussion

In this survey in Southern Benin, rabbit farmers were mostly men, rather middle-aged, educated, monogamous and owned in average 111 rabbits. A previous study on rabbit production and network in Benin (Kpodekon et al., 2000) makes similar observations about rabbit farmers' gender, age and education level, but 89% of them owned less rabbits (20 breeding does or less). This increase in the number of raised rabbits may be an indication of the intensification of the sector in Benin (Kpodekon et al., 2000). In Enugu State of neighboring Nigeria (Shah et al., 2018), the majority of rabbits producers are likewise men, but younger (20.37 years in average) and bachelor, with small rabbits farms as in previous reports (Onifade et al., 1999; Lukefahr 2007; Oseni et al., 2008). Indeed, rabbit production could be intensified to solve the problem of low animal protein intake in the developing world (Karikari and Asare 2009). This intensification requires professionalization and training of farmers in rabbit farming techniques, as well as hygiene and biosecurity. Lukefahr (2007) reported that formal rabbit farmer training, although usually taking place at a Research and Development training center, may be more appropriate when held on small demonstration farms in the region. This training is important to reduce some disease prevalence and improve production. Thus, training of farmers on basic rabbit management techniques, especially in smallholder units, is seen as a priority step in sustainable rabbit Research and Development programs. Several major publications (Finzi, 2008; Djago et al., 2007; Lukefahr, 2010) have laid out very comprehensive training steps in rabbit management under small-scale or backyard systems.

The practice of biosecurity measures is important to reduce the incidence of diseases on the farm. Biosecurity can be defined as a series of management practices designed to prevent, minimize, and control the introduction, spread, and release of diseases (Borrelli et

al., 2011). These measures include cleaning and disinfection of buildings and materials, use of footbath, manure handling, feed storage, disposal of dead animals and quarantine before introducing a new animal into the farm (UVM, 2010). The effectiveness of disinfection and use of footbath depends on frequency, prior cleaning and type of disinfectant according to the targeted germ. The recommended disinfectant against RHD are sodium hypochlorite, formaldehyde or substituted phenolic. Quaternary ammonium compounds are not effective against RHD calicivirus because it lacks the fatty envelope that most viruses have (APHIS/USDA, 2018; OIE, 2018b). Then, farmers in this study can be divided in two categories regarding biosecurity practices: groups 1 and 3 with 42.11% of farmers, where building and materials are cleaned and disinfected and footbath used with proper disinfectant respectively sodium hypochlorite and aldehydes or phenol; and group 2 with 57.89% of farmers, where building and materials are not disinfected and footbath not used. These observations are consistent with those of Ogolla et al. (2017) on farmer practices that influence prevalence of rabbit coccidiosis in Central Kenya with only 10.5% of farmers who cleaned the hutches with water and disinfectants. Even though others biosecurity measures like quarantine or disposal of dead rabbits had not been considered for the typology in this study, these measures are important in RHD prevention because RHD calicivirus is very resistant in the environment. Indeed, the virus can survive in chilled or frozen rabbit meat, as well as in decomposing carcasses or on cloths: 7 months in organ suspensions at 4° C, 3 months in the dried state at room temperature, 20 days at 22° C in decomposing rabbit carcasses or 2 days at 60° C in an organ suspension and the dried state. Outside the host, it can survive for 1 hour at 50° C, 3-7 days at 37° C or 22-35 days at 22° C (APHIS/USDA, 2018; OIE, 2018b).

In this study, vaccination was not relevant in the typology of rabbit farms. But about 1 in 10 farmers vaccinate against RHDV1 with an inactivated virus vaccine from the 3116-AP strain. Protection with this vaccine begins by day 6 after vaccination and lasts for 12 months. As RHD is endemic in Benin, the recommended protocol is vaccination of breeding rabbits from 2 months of age and revaccination once a year. Fattening rabbits are vaccinated only if the disease occurs, at weaning time (OIE, 2018b).

All the rabbits sampled in surveyed farms were free from anti-RHDV and anti-RHDV2 antibodies at the time of the study. This suggest that these rabbits or the mothers of young ones had never been in contact with the wild or the vaccine strain of the virus although RHD occurs in some farms between 2015 and 2018 and some farmers claim to vaccinate their rabbits. Indeed, maternal IgG antibodies from recovering or vaccinated

mothers persists for up to 8 weeks in young rabbits. Likewise, in rabbits that recover after infection, IgM and IgA titres rapidly reach a peak within 2 weeks after what IgM titres decline quickly while IgA persists more. IgG titres reach a peak more slowly after 15-20 days but persist for many months. However, in case of re-infestation, IgA and IgG titers can significantly rise again. In vaccinated rabbits antibodies response is quite the same except that no IgA is produced (Cooke *et al.*, 2000; OIE, 2018a).

In the other hand, RHD cases since 2015 seem to be higher in groups 1 and 3 that had good biosecurity practices than in group 2. The reason could be that farmers in both groups have adopted biosecurity measures because of the loss encountered in recent years. Finally, as farmers in group 2 were not highly educated compared with those in groups 1 and 3 we could also suppose that the more farmers are educated, the more they understand and practice biosecurity.

Conclusion

Biosecurity practices are important for disease prevention and management. This survey in rabbit farms in southern Benin Research and Development Units revealed difference in biosecurity practices among farmers. Paradoxically, farmers who had good biosecurity practices are the ones who encountered more VHD cases the past three years. Moreover, no antibodies against RHDV and RHDV2 had been detected in rabbits from study area suggesting that the sampled rabbits had never been vaccinated or in contact with the wild RHDV. Vaccination against VHD practice by farmer is therefore in question in this area and must be investigated.

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