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RETROSPECTIVE STUDIES ON RABBIT HAEMORRHAGIC DISEASE OUTBREAKS CAUSED BY RHDV GI.2 VIRUS ON FARMS IN FRANCE FROM 2013 TO 2018

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Abstract: Rabbit haemorrhagic disease (RHD) is a critical health threat to the rabbit industry in Europe. In 2018, the French rabbit industry adopted a voluntary control plan against this disease. In this context, two epidemiological studies were conducted on RHD outbreaks that occurred between 2013 and 2018 in France. The objectives were to describe the spread of RHD due to the new genotype RHDV GI.2 (rabbit haemorrhagic disease virus GI.2) and to identify rearing factors influencing the occurrence of the disease in order to guide the prevention measures recommended in the control plan. An analysis of cases on 295 farms between 2013 and 2017 showed that 32% of farms were affected at least once; the incidence of the disease increased in 2016-2017 compared to 2013-2015. Farms already affected in 2013-2015 had a higher risk of being infected in 2016-2017 than those that remained unaffected until 2015 (Relative Risk and 95% Confident Interval 1.7 [1.1-2.7]). A case-control study carried out between 2016 and 2018 on 37 outbreaks and 32 control farms revealed variability in biosecurity and decontamination practices between farms. The risk of being infected tends to be linked to these practices, but certain structural factors (e.g. the manure disposal system, transfer of rabbits at weaning) could also influence the risk of virus introduction into farms. In the context of a limited vaccination coverage of the farms (only females are vaccinated), these hypotheses will be studied further, using information from the RHD outbreak monitoring system implemented at the same time as the control plan in 2018.

Key Words: rabbit, haemorrhagic disease, epidemiology, outbreak.

INTRODUCTION

Rabbit haemorrhagic disease (RHD) is a highly contagious viral hepatitis that affects domestic and wild European rabbits (*Oryctolagus cuniculus*). This generally fatal disease is caused by a virus (RHDV) belonging to the *Lagovirus* genus of the *Caliciviridae* family. Since the emergence of the disease in China in 1984, it has spread worldwide to become endemic on rabbit farms in Europe, requiring systematic vaccination of breeding does, like for myxomatosis (*Abrantes et al., 2012*). Since 2010, a new viral genotype has appeared, first detected in France (Le Gall-Reculé *et al.,* 2011), originally named RHDV2 and now termed GI.2 (Le Pendu *et al.,* 2017). In 2019, Rouco *et al.,* reviewed the rapid spread of the GI.2 virus worldwide from France to southern Europe within one year (Italy, Spain and Portugal), then to northern Europe (United-Kingdom, Norway), North Africa, Oceania and North America over the period 2013-2018. This new genotype has almost replaced the classical RHDV (now termed GI.1) in several countries and is now the main genotype that circulates in livestock and wildlife, notably in Europe and in France (Le Gall-Reculé *et al.,* 2013). GI.2 virus has the specific feature of also infecting several hare species. This emergence has forced an adaptation of the RHD control strategy, with the development of new vaccines, as vaccines targeting GI.1 virus are not protective against infection by GI.2 virus. Despite the availability of several commercial vaccines, RHD remains a serious problem on commercial farms in France.

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In French rabbit farms, females but not kits are vaccinated against virus infection (Le Gall-Reculé and Boucher, 2017), as farmers cannot afford to vaccinate all the animals. However, GI.2 virus is known to infect young rabbits before 30 days of age, in contrast to the GI.1 virus (Dalton *et al.,* 2018; Le Gall-Reculé et *al*, 2013; Neimanis *et al.,* 2018). Under this condition, a vaccination strategy based on the vaccination of only reproductive does fails to completely prevent the spread of the disease.

Faced with this major health challenge, the French rabbit industry set up a national voluntary plan to contain RHD in 2018. This plan included a vaccination programme for reproductive does, increased biosecurity measures on farms and emergency vaccination of rabbits in the event of an outbreak. The control plan also included the implementation of a disease surveillance programme to identify outbreaks and strengthen biosecurity measures on neighbouring farms. However, implementing efficient control measures required precise knowledge of the epidemiology of the disease, particularly its pathways of introduction into rabbit farms. Pending the information that is to be collected as part of the monitoring of incident outbreaks, two retrospective studies were conducted on RHD outbreaks that occurred on farms in France between 2013 and 2018. The objectives of these preliminary studies were to describe the temporal evolution of the disease and identify structural factors or husbandry practices that influenced the occurrence of the disease. It included a five-year general retrospective study (2013-2017) and a case-control study focusing on outbreaks reported between 2016 and 2018.

MATERIALS AND METHODS

General description of the rabbit industry in France

France has about 800 commercial rabbit farms, mainly located in the west of the country (ITAVI, 2019). The vast majority of them are farrowing-to-finish farms, selling rabbits at around 11 to 12 wk of age.

Retrospective study on 2013-2017 outbreaks

Four rabbit veterinarians specialising in rabbit production agreed to take part in the five-year retrospective study. The target population (or at-risk population) for the survey was the rabbit farms monitored by the four practitioners over the studied period. The vets were accustomed to visiting these farms several times a year for routine check-ups or for urgent sanitary problems. The vets received two questionnaires consisting of Excel files: the first file was for the census of RHD outbreaks occurring in their rabbit farm practice population from 01/01/2013 to 31/12/2017. An outbreak was defined as a clinical case of RHD observed by the vets on the farm and confirmed based on laboratory analysis (RT-PCR for GI.2 virus on liver samples). The date of the clinical diagnostic was registered for each outbreak. In the second questionnaire, information on rabbit farms (including both infected and non-infected farms) was collected (Table 1). Only commercial rabbit farms (with more than 100 productive does) were included in the study.

Maps on the outbreak incidence per year were obtained using the “maptool” package from the R core team project. The annual risk of RHD was calculated as the number of new RHD outbreaks in the target population over a year, with a 95% binomial confidence interval. Only the first occurrence of RHD on a farm in a year was taken into account for the annual risk calculation (Dohoo *et al.,* 2003). As the target population was the farms monitored by the four vets, it was considered as a closed population, with no addition of farms over the 5-yr period and few withdrawals. A survival study on the target farm population was carried out using the non-parametric Kaplan-Meier method to generate the survival curve (survit function from the “survival” R package). The model used was a right-censored model, as all farms were included in the study at the same date (01/01/2013, no left-censored farms). The effect of farm and husbandry characteristics (size, type of housing, vaccination coverage) on the risk of RHD occurrence was tested using the log-rank test (survdiff function from the “survival” R package).

Case-control study (2016-2017)

Six rabbit veterinarians specialising in rabbit production agreed to participate in the case-control study on outbreaks occurring in 2016 and 2017. The case-control study was focused on the last two years to limit memory biases, in particular on the biosecurity measures in place at the time of the outbreak. Firstly, each veterinarian selected up to ten farms in his/her practice population, which were affected by an RHD outbreak in 2016 or in 2017. The criteria

Table 1: Data collected in the five-year retrospective study and in the case-control study.

Retrospective study 2013-2017	
Date(s) of outbreaks	
Number of productive does	
Type of housing: fixed cages / polyvalent cages (all-in / all-out)	
Open-air housing for growing rabbits: Y/N	
Case-control study	
All farms	Farm characteristics
	Type of farms: farrowing / farrowing and fattening
	Number of productive does
	Number of buildings
	Type of housing: fixed cages / polyvalent cages (all-in / all-out)
	Faeces collection system: deep pit / scraping system
	Open-air housing for growing rabbits: Y/N
	Husbandry practices
	Reproductive rhythm: 42 days / 49 days
	Number of batches per year
	Purchase of breeder does: Y/N
	Biosecurity
	Anteroom: Y/N
	Boot dip at the building entrance: Y/N
	Changing boots before entering for farmers: Y/N
	Changing clothes before entering for farmers: Y/N
	Changing boots before entering for visitors: Y/N
	Changing clothes before entering for visitors: Y/N
	Cleaning and disinfection programme
	Insect and rodent control programmes: Y/N, frequency
Cases	Date of the outbreak
	Infected animals: does / young does / kits
	Clinical signs
	Mortality rate
	Vaccination scheme before the outbreak: frequency, vaccine name
	Emergency vaccination: Y/N
	Disinfection measures

for defining outbreaks were the same as in the retrospective study over 2013-2017. Secondly, a control farm of a similar size and located in the same region (50-km radius) was matched to each case farm. A farm was considered a control farm if it did not experience an RHD outbreak in the last five years (2013-2017). A questionnaire (Table 1) was designed to collect general information on the selected farms and to describe RHD outbreaks on case farms. The vets filled in the questionnaire and the farmers were contacted by telephone if the vets could not complete certain questions. The differences in farm characteristics and husbandry practices between case and control farms were assessed using non-parametric tests (catdes function of the FactomineR R package). The diversity of farm characteristics and husbandry practices was explored using a factorial analysis (factorial analysis for mixed data, FAMD), followed by a hierarchical clustering on principal components (HCA) to constitute classes of farms with similar practices (FAMD and HCPC functions of the FactomineR R package). At this point, the status of the farms (case or control) was included in the analysis as an illustrative variable.

RESULTS

Retrospective study on 2013-2017 outbreaks

The practice population of the four vets taking part in the study was made up of 295 rabbit farms in 2013. Five farms stopped producing rabbits during the period studied, but it was not possible to establish whether this was linked to

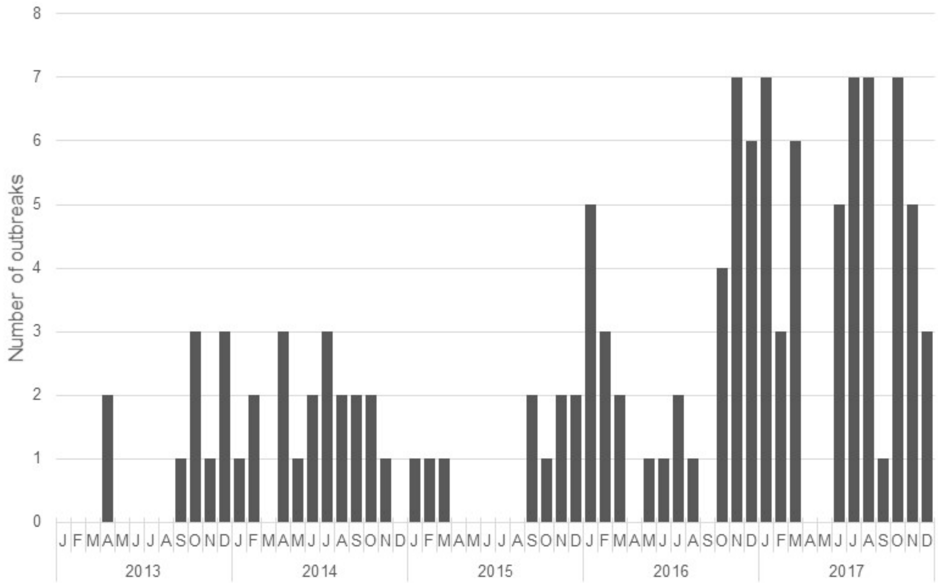

Figure 1: Number of RHD outbreaks per month from 2013 to 2017 (N=119 outbreaks, 295 monitored farms, France). The month reported is the month of diagnosis of the first RHD outbreak on a farm if several episodes occurred in the same farm and in the same year.

an RHD outbreak or not. Over the five-year period, 94 farms (32%) were affected by at least one RHD outbreak. In total, 119 outbreaks were observed, with 74 farms affected once (74/295, 25%), 15 farms twice (15/295, 5%), and 5 farms affected 3 times (5/295, 2%). No seasonal pattern could be detected in the monthly incidence over the studied period (Figure 1). The risk of a farm being affected by RHD over a year increased in 2016 and 2017 in comparison with the previous three years (Table 2). This increased risk was observed in all areas covered by the study (Figure 2).

Due to the observed increase in occurrences of RHD, the studied period was divided into two separate periods, from 2013 to 2015 (the first three years) and from 2016 to 2017 (the last two years). During the first period, 37 farms faced at least one RHD outbreak and 257 remained non-affected. From 2016 to 2017, one or two RHD outbreaks occurred on 71 farms, including 14 farms that had been infected during the first period (farms with "antecedent outbreaks"). The risk for a farm with an "antecedent" of being affected over the 2016-2017 period was 38% (14/37), while this risk was 22% (57/258) for farms without an "antecedent". Therefore, the relative risk of being infected in 2016-2017 was 1.7 ($CI_{95\%}$, [1.1-2.7]) for farms with an "antecedent", in comparison with non-affected farms during the 2013-2015 period (P=0.03).

Table 2: Number of outbreaks and annual risk of RHD on rabbit farms from 2013 to 2017 (N=295 farms, France).

Year	Number of farms under study	Number of outbreaks	Annual risk	$CI_{95\%}$
2013	295	10	0.034[a]	[0.013-0.055]
2014	295	19	0.064[a,b]	[0.036-0.092]
2015	293	10	0.034[a]	[0.014-0.055]
2016	293	31	0.106[b,c]	[0.07-0.14]
2017	290	49	0.169[c]	[0.124-0.208]

[a,b,c] Risks with different superscripts differ at P<0.05.

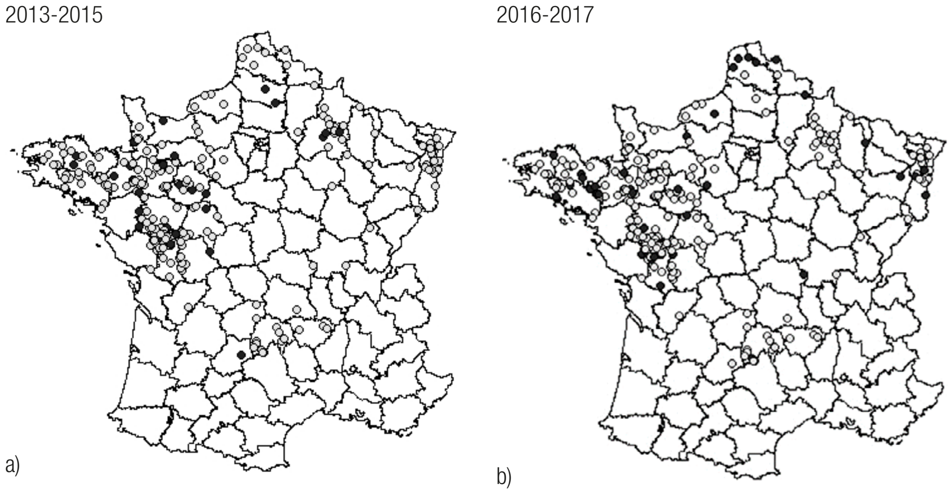

Figure 2: Geographical distribution of rabbit haemorrhagic disease (RHD) outbreaks in a) 295 farms monitored from 2013 to 2015 and b) 293 farms monitored from 2016 to 2017. ●: Farm with outbreak; ○: Farm without outbreak.

Data on farming conditions were obtained for 81 farms, including 30 affected by RHD at least once from 2013 to 2017 (Table 3). The Kaplan-Meier survivor function for that subset of farms is shown on Figure 3. The size of the farm, the type of housing or open-air housing did not influence the survival probability of the farms based on the log-rank test results.

Case-control study (2016-2018)

The study involved 37 case farms and 35 control farms. Two control farms could not be contacted by telephone to complete the questionnaire. The RHD outbreaks occurred from March 2016 to July 2018. The disease first and mainly affected kits after weaning (average age=50 d) on 31 farms out of 37, but kits before weaning were also infected on three farms. The mortality rate of weaned rabbits varied from 10% up to 80% (average 31%). However, the mortality rate associated with the RHD outbreaks was seldom reported (25/37 farms), as mortality counting was sometimes stopped during the outbreak. On six farms, the disease affected females only, with a mortality rate varying from less than 1% to 30%. At the time of infection, six farms did not vaccinate reproductive does against RHD. Emergency vaccination was practiced on reproductive does (full dose) on 13 farms and on weaned rabbits (half dose

Table 3: General characteristics and risk of RHD of farms enrolled in the retrospective 5-year study (N=81 farms with data, France, 2013-2017).

	No. farms	No. farms affected by RHD[a]	% affected farms	P χ^2-test
Number of productive does				
]100-400]	15	4	27	0.10
]400-800]	45	19	42	
> 800	10	7	70	
Type of housing				
fixed cages	19	6	32	0.53
polyvalent cages (all-in / all-out)	62	24	39	
Open-air housing for growing rabbits				
yes	13	5	38	0.91
no	68	25	37	

[a]at least once over the studied period.

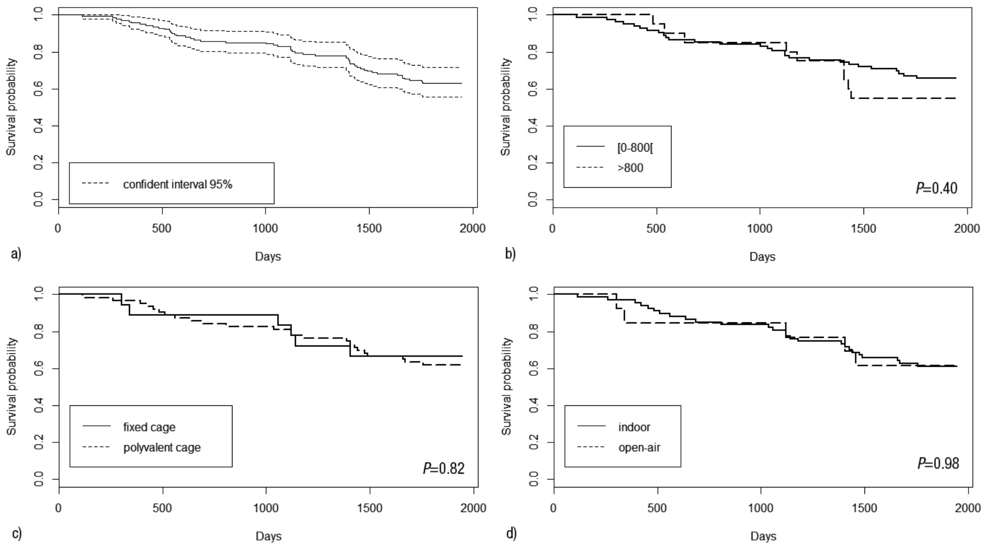

Figure 3: Survival curves and log-log rank test probabilities a) for the overall population, and according to b) number of productive does, c) type of cage, and d) type of housing (N=81, France, 2013-2017).

due to cost limitations) on 24 farms. Complete cleaning (with detergent) and disinfection were applied on 20 infected farms, but slurry was treated with lime (the only product used) before land spreading on 6 farms only.

Among all the farm characteristics studied, only one of them was associated with a higher risk of RHD infection (Table 4): the risk was higher when the farmers did not follow a well-defined cleaning and disinfection programme during the downtime period between rabbit batches (Odds Ratio=3.7, [1.1-13.0], *P*=0.03). The multidimensional analysis (FAMD and HCPC) made it possible to distinguish three classes of farms based on their structural characteristics and farming practices (Figure 4). A first class of farms (class 1, N=27) was characterised by high standards for the cleaning and disinfection programme; these farms often had more than two rabbit houses and transferred the kits at weaning to a specific growing and finishing house. Most of the houses were equipped with a deep pit to collect slurry. On the contrary, the second class of farms (N=26) grouped together farms with two rabbit houses, with polyvalent cages (transfer of female does at weaning) and a slurry scraping system. These farms were more often equipped with a sanitary anteroom, a boot dip at the house entrance, but did not apply a complete cleaning and disinfection programme. The last category of farms (N=19) did not apply specific biosecurity measures. It is of interest to point out that the frequency of RHD cases tended to be lower in class 1 (10/27, 37%) than in class 3 (12/19, 63%, *P*=0.08); the difference in case frequencies was not significant between class 1 and class 2 (15/26, 58%, *P*=0.13).

DISCUSSION

Little knowledge is available on the epidemiology of RHD due to GI.2 virus on industrial rabbit farms in Europe. Carvalho *et al.* (2017) described the course of the infection on a farm where emergency vaccination was applied. Rosell *et al.* (2019) recently published a 30-yr retrospective survey on occurrences of RHD in commercial farms in Spain. Nevertheless, most of the communications are limited to reports on the detection of GI.2 virus in a new geographical area. However, a description of the RHD dissemination within the rabbit industry is a prerequisite for assessing the impact of the disease on the production sector, guiding strategic choices for the implementation of a control plan and dimensioning the necessary resources for this purpose. The practical objective of our studies was to use retrospective data directly available from vets to assist in the implementation of the national control plan. Four vets from the rabbit branch of the National Veterinary Association (SNGTV) agreed to take part in the five-year retrospective study. The sample of farms under study accounted for approximately one third of the national number of rabbit farms

Table 4: Farm characteristics, husbandry practices and biosecurity measures on RHD-infected farms (cases) and control farms (France, N=72 rabbit farms, 2016-2018).

		Cases (N=37)		Controls (N=35)		
		n	%	n	%	*P*
Production and housing	Number of reproductive does					
	< 400	17	46	16	46	0.98
	[400-800[	10	27	10	28	
	≥ 800	10	27	9	26	
	Reproductive rhythm					
	42 day rhythm	32	86	33	94	0.26
	49 day rhythm	5	13	2	6	
	Number of doe batches					
	One batch	36	97	30	86	0.08
	Two or three batch	1	3	5	14	
	Purchase of 1-day old females					
	Yes	10	27	13	37	0.36
	No	27	73	22	63	
	Purchase of 10-day old females					
	Yes	3	8	8	23	0.08
	No	34	81	27	77	
	Grand-parental line					
	Yes	24	65	18	51	0.25
	No	13	35	17	49	
	Number of rabbit houses					
	One	20	54	15	43	0.60
	Two	12	32	13	37	
	Three or four	5	14	7	20	
	Type of cages					
	Fixed cages (kit transfer at weaning)	13	35	17	49	0.25
	Polyvalent cages (doe transfer)	24	65	18	51	
	Open-air housing for growing and finishing					
	Yes	10	27	8	23	0.25
	No	27	63	27	77	
Slurry management	Slurry collection system					
	Deep pit	11	30	13	37	0.50
	Scraping system	26	70	22	63	
	Equipment for slurry spreading					
	Specific to the farm	10	27	6	17	0.31
	Common with other farms	27	63	29	84	
	Surface spreading system					
	Yes	13	35	11	31	0.95
	No	24	65	24	69	
	Cleaning of spreading equipment					
	Yes	12	33	15	43	0.36
	No or not known	25	67	20	57	
	Spreading of rabbit slurry near the farm					
	Yes	11	30	8	23	0.40
	No	26	70	27	77	

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		Cases (N=37)		Controls (N=35)		
		n	%	n	%	*P*
Biosecurity measures	Access road closed with a barrier or a chain					
	Yes	15	40	13	37	0.77
	No	22	60	22	63	
	Car park for visitor cars					
	Yes	21	57	20	57	0.97
	No	16	43	15	43	
	Trucks park near air entrances					
	Yes	14	38	12	34	0.75
	No	23	62	23	66	
	Rendering truck enters the farm					
	Yes	10	27	13	37	0.36
	No	27	73	22	63	
	Distance from the rabbitry to the carcass container					
	Less than 50 m	21	57	25	72	0.19
	More than 50 m	16	43	10	28	
	Boot dip					
	Yes	25	60	28	80	0.23
	No	12	40	7	20	
	Sanitary anteroom					
	Yes, three-zone anteroom	12	32	8	23	0.49
	Yes, two-zone anteroom	16	43	7	20	
	No	9	24	20	57	
	Hand-washing equipment					
	Yes	22	59	25	72	0.29
	No	15	41	10	28	
	Farmers wear specific footwear into the rabbitry					
	Yes	30	81	31	88	0.38
	No	7	19	4	12	
	Farmers wear specific clothes into the rabbitry					
	Yes	31	89	32	91	0.33
	No	6	11	3	9	
Cleaning and disinfection	Cleaning and disinfection programme					
	Yes	25	59	31	88	0.03
	No	12	41	4	12	
	Use of detergent for cleaning					
	Yes	9	24	13	37	0.24
	No	28	76	22	63	
	Anteroom cleaning and disinfection					
	Yes	19	51	18	51	0.99
	No cleaning or no anteroom	18	49	17	49	
	Disinfection of concrete areas					
	Yes	24	65	28	80	0.15
	No	13	35	7	20	
	Disinfection of carcass container					
	Yes	19	51	20	56	0.62
	No	18	49	15	43	

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		Cases (N=37)		Controls (N=35)		
		n	%	n	%	*P*
Cleaning and disinfection	Disinfection of feed silo					
	Regular (several times per year)	6	16	5	17	0.82
	No regular disinfection	31	84	30	83	
	Disinfection of deep pit or slurry pit					
	Regular (several times per year or when empty)	9	24	6	17	0.45
	Less often or not done	28	76	29	83	
	Sanitary downtime period					
	One day or less	15	40	14	40	0.56
	Two or three days	14	38	11	31	
	Four days or more	8	22	10	29	
Wildlife control, domestic animals and surroundings	Farmers own a backyard					
	Yes	27	63	26	74	0.88
	No	10	27	9	26	
	Farms enclosed with fences					
	Yes	8	22	2	6	0.07
	No	29	78	33	94	
	Professional rabbit farms near the farm					
	Yes	15	40	9	26	0.18
	No or not known	22	60	26	74	
	Backyards with rabbits near the farm					
	Yes	19	51	25	72	0.06
	No or not known	18	49	10	28	
	Wild rabbits and/or hares near the farm					
	Yes	21	57	25	72	0.20
	No	16	33	10	28	
	Foxes and badgers near the farm					
	Yes	14	46	20	57	0.10
	No	23	54	14	43	
	Frequency of wildlife occurrence near the farm					
	Frequent occurrences	12	32	16	46	0.17
	Rare occurrences	16	43	16	46	
	No wildlife seen near the farm	9	25	3	8	
	Contract for rodent control					
	Yes	19	51	16	46	0.63
	No	18	49	19	54	
	Farmers practice hunting					
	Yes	4	11	4	11	0.93
	No	33	89	31	89	

in France, estimated to be around 800 farms by the French professional corporation (FENALAP) in 2017. As the farm sample was constituted by convenience, we tested whether it was representative of the general population of rabbit farms by comparing its characteristics to the results of the annual survey RENACEB on economic performance of rabbit farms (N=749 farms monitored in 2015, Hurand *et al.,* 2016). About 75% of the farms in our survey were located in the western part of France compared to 80% of the farms in the RENACEB survey. The average size of the farms was 625 reproductive does in our sample versus 619 in the RENACEB survey. Data on the housing system was available for 81 farms in the retrospective study: 76% of those farms had polyvalent cages (kits stay in the cage after weaning) in contrast to the 50% in the RENACEB survey. It is unclear whether this difference might have led to a bias in the estimate of annual disease incidence, as cage type was not identified as a risk factor for the disease in the case-control study.

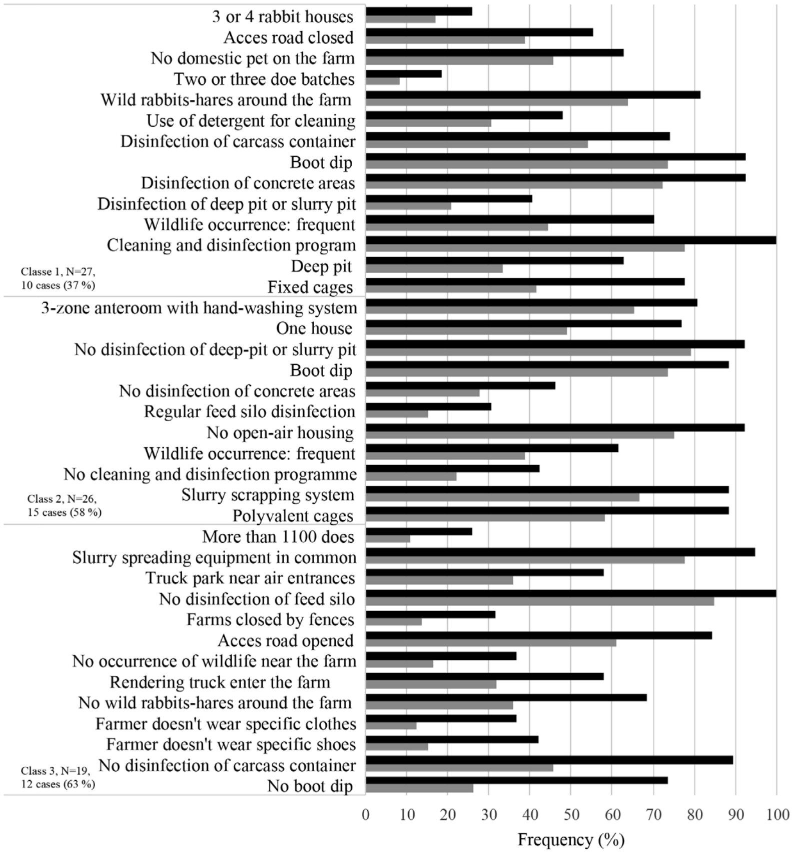

Figure 4: Classification of farms according to husbandry practices, biosecurity measures and environmental conditions (N=72 farms, France, 2016-2018). Modalities within a class are ordered according to *P*-values (from significant $P<0.05$ to highly significant $P<0.001$). ■ in the class; ■ in the overall sample (N=72).

The results of the retrospective survey made it possible to identify a change in the epidemiology of the disease, with an increase in the incidence of cases since 2016. This increase was also noted in the rabbit and wild hare populations: data from the SAGIR surveillance network on diseases in wildlife showed an increase in grouped deaths due to RHD between 2014-2015 (97 confirmed cases) and 2016-2017 (181 cases) in France. Such an increase in RHD incidence was not observed in Spain (Rosell *et al.,* 2019). According to this survey, an increase in occurrence of RHD outbreaks was observed twice in Spain: when no vaccine was available against GI.1 in 1988-1989, and when the GI.2 virus appeared in 2011-2013. The determinants of the 2016-2017 increase in France are not known. Recent studies proved that the vaccines used on farms provide sufficient protection against the GI.2 strains found in the field (Carvalho *et al.,* 2017, Le Minor *et al.,* 2017, Le Minor *et al.,* 2019). The increase in RHD prevalence in the 2016-2017 period might be linked to an increase in virus virulence as suggested by Capucci *et al.* (2017). However, it is also important to note that our study did not take into account vaccination practices, such as the rhythm of vaccination for females. Many factors in the use of vaccines can also influence their effectiveness, apart from a change in virus characteristics. The more favourable situation towards GI.2 virus in Spanish farms (Rosell *et al.,* 2019) than in French farms could be linked to a better vaccination coverage.

The case-control study conducted over the period 2016-2018 did not reveal key risk factors primarily influencing the risk of a farm being infected by RHD. However, the results highlighted a wide diversity in the implementation of biosecurity measures on rabbit farms: some farms did not apply biosecurity measures (class 3), some farms were well-equipped with control barriers but did not apply a complete cleaning and disinfection programme (class 2). Class 1 farms paid more attention to decontamination procedures. Biosecurity remains a key element in RHD control: wild rabbits are a proven reservoir of viruses, which can also be carried by insects (Mc Coll *et al.,* 2002; Hall *et al.,* 2019). In addition, passive contamination (material, human activities, predators and scavengers) might have an important role in virus spreading (Cooke and Fenner 2002). Only a detailed study of biosecurity practices and, above all, of their application over time, could make it possible to demonstrate their impact on the risk of contamination. Nevertheless, RHD cases tended to be less frequent in class 1 farms than in the two others. Two hypotheses may be considered to explain this observation. On the one hand, the class 1 farms applied strict cleaning and disinfection procedures, including decontamination of rendering containers and concrete areas around the rabbit houses. The farmers also closed the access road to the farm with a fence or with a chain. These procedures might limit the risk of RHDV introduction in the farm by wildlife and vehicles; the risk of viral persistence in the environment is also reduced by regular disinfection. On the other hand, class 1 farms showed some specificities in their husbandry management and building conception: three or more rabbit houses, deep pits and fixed cages. Deep pits are emptied every year and a half, or every two years, and are disinfected on this occasion. The rest of the time, these pits are sealed, which limit the risk of wildlife introduction in comparison with slurry scraping systems (with manholes in the house walls for slurry evacuation).

The higher risk of infection on farms that have already experienced RHD outbreaks suggests that certain structural factors might increase the risk of GI.2 virus introduction on farms, as reported in the case-control study. In addition, persistence of RHD virus on an infected farm and its environment cannot be ruled out. Rabbit infection by GI.2 virus leads to high levels of virus in tissues and in faeces (Matthaei *et al.,* 2014, Dalton *et al.,* 2018). The RHD virus can remain viable several weeks in tissues and the environment (Henning *et al.,* 2005).

Our epidemiological studies aimed to collect preliminary descriptive and explicative information on the RHD epizootics on rabbit farms in France in order to assist in the implementation of the national control plan. The complementarity of the two epidemiological studies has made it possible to draw conclusions on the general evolution of the disease in a large population of farms and to provide initial detailed information on the farming factors influencing the occurrence of the disease. Analysing data collected by the vets in their daily practice confirmed the specific evolution of the RHD in French farms marked by the increase of outbreaks in 2016 and 2017. Farms infected at the beginning of the epizootic in 2013-2015 showed a higher risk of infection in 2016-2017 than the others; further studies are needed by to understand the specific characteristics of these "at-risk" farms. Reinforcement of vaccination on these farms is strongly recommended. The case-control study showed high diversity in cleaning and disinfection practices and biosecurity measures on rabbit farms. Good cleaning and disinfection practices were associated with a lower risk of infection. In the context of an incomplete vaccination strategy in French farms, a complete programme of cleaning and disinfection, including the use of detergent products and treatment of surrounding areas on the farm, is therefore a key practice to promote for the control of RHD.

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