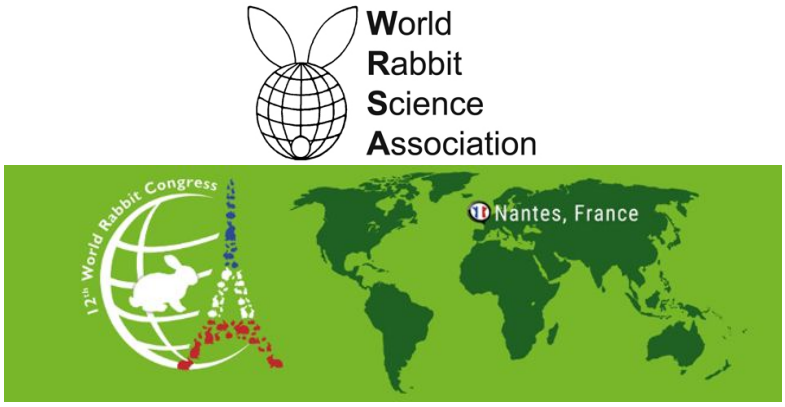

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EVALUATION OF CLEANING AND DISINFECTION PROCEDURES IN RABBIT FARMS AFFECTED BY RABBIT HAEMORRHAGIC DISEASE, IN FRANCE

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ABSTRACT

Rabbit Haemorrhagic Disease (RHD) affects many commercial rabbit farms in France. Some farms experience several successive outbreaks, which raises the question of the efficacy of cleaning and disinfection (C&D) measures implemented after the outbreaks. This study aimed to evaluate the efficacy against RHD virus of C&D protocols applied on four infected farms in 2019. We sampled the husbandry rooms and their surroundings by swabbing to detect the RHDV2 genome by RT-PCR. Samples were taken before C&D, after C&D and three month later. A total of 35 samples out of 75 taken before C&D were positive for RHDV2 (47%). The most frequently contaminated surfaces were the rendering container (3/4), the floor of the husbandry room (3/4) and the surroundings (4/6). Virus genome was thus detected on equipment in contact with rabbits but also on surfaces soiled by faeces, blood and dust. After C&D, the RHDV genome was detected in 14 samples out of 74 (19%). The rendering containers were positive on three farms: they had not been treated during C&D operations. Three months later, RHDV genome was still recovered from rendering containers on two farms. Residual contamination may be observed after decontamination in insufficiently treated areas. This underlines the importance for the farmer and the technical advisors to establish a complete decontamination protocol adapted to the farm.

Key words: Rabbit Harmorrhagic Disease, Disinfection, Cleaning, Rabbit

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. According to the results of the surveillance system set up by the rabbit sector in France in June 2018, the disease affected approximately 135 commercial farms in one year, out of a total estimated population of nearly 800 farms. Of these farms, 49 had already been infected at least once with the disease. This raises the question of whether the virus can be maintained in an affected farm and possibly cause a new outbreak. The RHD viruses are indeed very resistant, remaining viable several weeks in tissues of dead animals and the environment (Henning et al., 2005).Thus, rabbits can be indirectly infected through contaminated food, water, clothing, equipment or vector-borne transmission (Abrantes et al., 2012). Cleaning and disinfection (C&D) are therefore important steps to eradicate the disease from the farm. The objective of our study was to evaluate the efficacy of decontamination procedures implemented in RHD outbreaks by monitoring the persistence of the virus in surface samples before and after C&D. During RHD outbreaks, effectiveness of disinfection

implies that the residual load of infectious RHDV2 particles on the treated surface is lower than the minimal infectious dose. Such references do not exist for the indirect transmission of RHD via a soiled surface. An alternative strategy is to use environmental sampling coupled with RHD genome detection by reverse-transcription polymerase chain reaction (RT-PCR) method. A positive result denotes the presence of RHDV genome but does not inform about virus viability or capacity of infection. Nevertheless, this type of protocol showed its interest for monitoring the effectiveness of control measures taken for other animal diseases as Avian Influenza (Kang et al., 2015). The present paper shows the results obtained on four outbreaks followed for 3 months.

MATERIALS AND METHODS

This observational study aimed to compare frequencies of RHDV2 genome detection on infected farms before and after decontamination. Three visits for sampling were carried out by farms from February to June 2019. The first visit took place within the two weeks following the outbreaks, before cleaning and disinfection (C&D) of the premises. The second and the third visits were carried out after the final disinfection and three months after respectively. C&D protocols tested were those applied by the farmers. Information about the C&D protocols was collected in a questionnaire filled in during the visits at the farm.

Up to 20 environmental samples were performed per visit. Samples were obtained from the contaminated premise from its direct surroundings. Surfaces were sampled with a fabric swab (swab N°4023, Sodibox, Nevez, France). Floor and neighbouring area were sampled using boot swabs (swab N°4130, Sodibox, Nevez, France) by walking for 3 minutes. At each visit, six samples were taken on walls and floor of the room, four samples on cages, two in the anteroom, four on the air system (cooling, fans etc.) and two on the slurry scrapping system. Two samples by boot swabs were obtained by walking on concrete areas around the building and on the road to the building. Samples were stored at 4°C and transported to the laboratory within 4 hours. Swabs were wetted with Saline buffer (PBS) and homogenized using an automatic paddle blender (BagMixer®) for 1 min. Total RNAs were extracted from 200 µL of liquid using the NucleoMag®VET kit (Macherey-Nagel, KingFisher instrument). One-step reverse transcriptions and amplifications were performed using lagovirus-specific primers and SuperScriptTM III One-Step Plantinium Taq HiFi (Invitrogen). PCR products were analyzed by electrophoresis on agarose gel.

RESULTS AND DISCUSSION

The four farms enrolled in the study were farrowing-to-finishing rabbit farms located in the western part of France. The farms had 200 to 800 reproductive does (median 665 does) in a single batch (3 farms) or in three batches (1 farm). In all farms, females were transferred to another part of the farm at weaning and the litters remained in the cages where they were born. All the rabbit premises studied were classical buildings (aged from 12 to 27 years, median 20), with a scrapping system for daily disposal of slurry. In the first farm studied, RHD occurred in January 2019 when the farm had never been affected before; rabbits does and their litter were infected but not the finishing rabbits. The last three farms had been already affected by RHD in 2018. The disease occurred in April (1 farm) and May 2019 (2 farms) and it affected rabbits during finishing (aged from 46 to 63 days, median 55 days).

Protocols for C&D are shown in Table 1. Rooms were cleaned and disinfected after that the finishing rabbits were sold but in one farm, some rabbits remained in a part of the room during C&D. The protocols used to clean the rooms in the four farms were very close, except in one farm where no detergent was used. Quaternary ammonium compounds (QACs) associated with aldehydes were the most used disinfectant products. Farmers preferred foaming products, which makes it easier to control the application

of the product. They were able to report the concentrations of detergent and disinfectant products used by foaming but they did not report clearly for the doses for products used by soaking (immersion), by fogging or by thermonebulization. Nests were dismantled and cleaned separately from the building.

Table 1: Cleaning and disinfection protocols applied in four RHD outbreaks

	Farm A	**Farm B**	**Farm C**	**Farm D**
		Cage and building		
Dry cleaning	**yes**	**yes**	**yes**	**yes**
Soaking	foam product alcalin detergent at the recommended dose 24-h contact time	foam product alcalin detergent at the recommended dose 30 min-contact time	no	foam product at doubled dose 30 min-contact
Washing	high-pressure washing with water at ambient temperature		high-pressure washing with warm water	high-pressure washing with water at ambient temperature
Disinfection 1	foam product QACs and gluteraldehyde solution at the recommended dose		foam product QACs, formalin and gluteraldehyde solution at the recommended dose	foam product QACs and gluteraldehyde solution at doubled concentration
Disinfection 2	fogging Phenylphenol and glycolic acid solution at the recommended dose 7 days after the 1st disinfection	thermonebulization QACs and gluteraldehyde solution 1 day after the 1st disinfection	foam product QACs, formalin and gluteraldehyde solution at the recommended dose 1 day after the 1st disinfection	foam product QACs and gluteraldehyde solution at doubled concentration 7 days after the 1st disinfection
		Nest		
Soaking	no	immersion in solution alcalin detergent at the recommended dose	immersion in solution QACs and gluteraldehyde solution The farmer did not know the concentration used 24 h-contact time	foam product at doubled dose 30 min-contact time
Washing	high-pressure washing with water at ambient temperature	high-pressure washing with water at ambient temperature	high-pressure washing with warm water	high-pressure washing with water at ambient temperature
Disinfection	foam product QACs and gluteraldehyde solution at the recommended concentration	no	immersion in solution chlorine solution. The farmer did not know the concentration used	foam product QACs and gluteraldehyde solution at doubled concentration
Storage	outdoor on a concrete area	outdoor on a concrete area	outdoor on a concrete area	outdoor on a concrete area
Placement in the building	before 2nd disinfection	after 2nd disinfection	after 2nd disinfection	after 2nd disinfection

At the first visit (8 to 15 days after the outbreak), 35 samples out of 75 (47%) were positive for RHDV2 genome, on farms A, B and D; no positive sample was obtained on farm C (Table 2). The most frequently contaminated surfaces were the rendering container (3/4), the floor of the husbandry room (3/4) and the surroundings (4/6). Virus genome was thus detected on equipment in contact with rabbits (cages, rendering container) but also on surfaces soiled by faeces, blood and dust (floor, walls, air system, and anteroom).

After C&D (visit 2, 3 to 30 days after disinfection), fourteen positive samples (out of 74, 19%) were observed, with respectively 9, 3, 2 and 1 positive samples on farms A, B, C and D. In farm A, walls, floor, ventilation fans and the anteroom were still positive. Indeed Farm A was the most heavily contaminated

Table 2: Detection of RHD genome before and after cleaning and disinfection

Location	Before C&D		After C&D		Three months later	
	Not detected	Detected	Not detected	Detected	Not detected	Detected
air system	11	4	12	2	16	
wall	10	8	18	1	18	
floor	1	3	2	1	4	
cage	8	8	14	2	16	1
anteroom	4	4	6	2	8	
scrapping system	3	1	3	2	7	
surroundings	2	4	5	1	4	1
rendering container	1	3		3	2	2
Total	**40**	**35**	**60**	**14**	**75**	**4**

before C&D. The rendering containers were positive on three farms: they had not been treated during C&D operations. Nests were cleaned and disinfected separately from the building and were placed in the cages after the second disinfection on three farms. As a result, nests in farm B were not disinfected when reused. However, no residual genome detection was observed as the disease occurred on the farrowing rabbits only. On farm A, an additional sample was taken on the nests that were stored outside the building, before replacement. RHDV2 genome was detected on that sample. At the second visit after C&D, two samples taken on cages with nests were positive on that farm. This observation underlined that nests may be a source of residual contamination if they are not decontaminated properly or stored in a clean closed building before being reused.

At the third visit (3 months after the outbreak), RHDV2 genome was detected on the rendering container in farms C and D and on the road near the rabbit premise in farm C. These observations are in accordance with those of Henning et al (2005) on classical RHDV. In their experiment, RHDV GI.1 can be isolated from animal tissues for at least 90 days and the virus was still infectious for rabbits. The detection of viral genome outside rabbit buildings shows that decontamination must include the entire breeding site. The impact of this residual contamination is difficult to assess. The monitoring of the farms over time will make it possible to determine, in the event of a new outbreak, whether the virus strain implicated in the new outbreak is the same as the one isolated after decontamination in the surroundings.

CONCLUSIONS

This observational study is the first to characterize the effectiveness of decontamination protocols applied in farms contaminated by RHD. Results show that the viral genome can be detected in different areas of the farm before decontamination. Residual contamination may be observed after decontamination in insufficiently treated areas. This underlines the importance for the farmer and the technical advisors to establish a complete decontamination protocol, including surroundings and rendering container, adapted to the farm.

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Evaluation of cleaning and disinfection procedures in rabbit farms affected by Rabbit Hemorrhagic Disease in France

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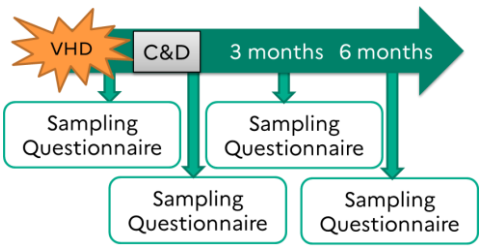
Rabbit Haemorrhagic Disease (RHD) affects 15 to 20% of the rabbit farms in France yearly since 2018.
Some farms experience several outbreaks, which raises the question of the efficacy of cleaning and disinfection (C&D) measures implemented after the outbreaks.

Take home message

- ❑This observational study is the first to characterize the effectiveness of C&D protocols applied in farms infected by RHD
- ❑The viral genome can be detected in different areas of the farm before C&D
- ❑Residual contamination may be observed after C&D in insufficiently treated areas
- ❑It is highly recommended to establish a complete decontamination protocol, including the surroundings of the farm and the rendering container

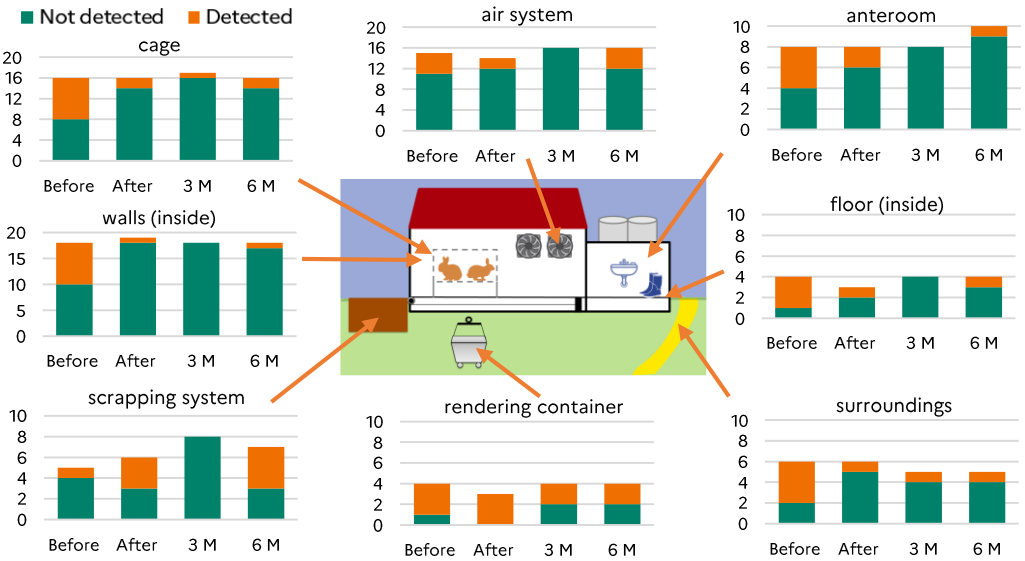
The objective of our study was to evaluate the efficacy of decontamination procedures implemented in RHD outbreaks by monitoring the persistence of the virus in surface samples before and after disinfection

A longitudinal study



- Four with RHD outbreaks enrolled in 2019
- Four visits for sampling and for filling a questionnaire about C&D
- 20 environmental samples per visit
- Samples analysed by one-step RT-PCR for RHDV2 genome detection

Detection of RHD genome by RT-PCR on environmental samples



The authors are grateful to Mr Gilles Urien and to the farmers
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