Avian influenza outbreaks: evaluating the efficacy of cleaning and disinfection of vehicles and transport crates

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ABSTRACT In 2021, France faced large avian influenza outbreaks, like in 2016 and 2017. Controlling these outbreaks required the preventive depopulation of a large number of duck farms. A previous study in 2017 showed that the quality of decontamination of trucks and transport crates used for depopulation was often insufficient. A new study was then set up to evaluate cleaning and disinfection (C&D) of trucks and crates used for duck depopulation and whether practices had changed since 2017. Three methods were used to assess decontamination: 1) detection of avian influenza virus (AIV) genome, 2) visual inspection of cleanliness, and 3) microbial counts, considering that 2 and 3 are commonly used in abattoirs. Another objective of the study was to evaluate the correlation between results obtained with the 3 methods. In 5 abattoirs, 8 trucks and their crates were sampled by swabbing to detect AIV genome by rRT-PCR before and after decontamination. Visual cleanliness scores and coliform counts were also determined on crates after C&D. Trucks and crates were decontaminated according to the abattoirs' protocols. Before C&D, 3 quarters of crates (59/79) and 7 of 8 trucks were positive for AIV genome. C&D procedures were reinforced in 2021 compared to 2017; use of detergent solution and warm water were more common. Nevertheless, 28% of the crates were positive for AIV genome after C&D, despite the fact that cleaning scores and microbiological counts were satisfactory for 84% and 91% of the crates, respectively. No correlation was observed between results for AIV genome detection and results from visual control or from coliform counts. Abattoirs are encouraged to use environmental sampling coupled with AIV genome detection to monitor the quality of cleaning and disinfection of trucks and crates during AI outbreaks. Reinforcement of biosecurity measures at abattoirs is still needed to avoid residual contamination of the equipment and cross-contamination during the decontamination process.

Key words: avian influenza, transport, cleaning, disinfection, biosecurity

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INTRODUCTION

Europe has experienced a widespread epizootic of highly pathogenic avian influenza (**HPAI**) both in wild birds and in poultry since October 2020. Regarding this outbreak in domestic poultry, more than 400 outbreaks occurred in France from November 2020 to April 2021 (Supplementary material 1). Most of the outbreaks occurred in the southern part of France and affected ducks reared for foie-gras production. To prevent spilling of the infection from the outbreaks, duck flocks were

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depopulated over a large area of 20 km radius around the outbreaks. Consequently, more than 3.5 million ducks were culled within 6 wk, from January to February 2021. Four abattoirs were involved in duck culling, along with a specific temporary facility. This open-air facility was set up on a platform usually used for cleaning and disinfection of the trucks transporting animals. The platform was located at the center of the infected area. It could process up to 20,000 birds per day (gaseous euthanasia). Birds to be culled were transported to the abattoir or the temporary platform in plastic crates on trucks covered with nets or plastic sheets. Afterwards, crates and trucks used to move birds were to be cleaned and disinfected (European Council Directive 2005/94/ EC).

A similar strategy of preventive culling was applied previously to control the HPAI epizootic that affected the duck farms in the same area during winter 2016

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Table 1	. Numb	per of	sample	s (swał	os and	stick-s	swabs) taken	for a	avian	influenza	virus	genome	detection	on	transport	crates a	and t	truck	٢S
(France	, 2021).																			

		_]	Before C&D		After C&D					
			Truck					Truck			
Site	Number of trucks	crate	$outside^1$	wheel	cabin^2	Total	crate	outside	wheel	cabin	Total
A	2	19	2	2	2	25	20	2	2	2	26
В	2	20	2	2	2	26	20	2	2	2	26
С	2	20	2	2	2	26	20	2	2	2	26
D	1	10	1	1	1	13	10	1	1	1	13
E	1	10	1	1	1	13	10	2	1	1	14
Total	8	79	8	8	8	103	80	9	8	8	105

¹Truck bed and rocker panels.

²Steering wheel, gear lever, and handles.

-2017. A previous study demonstrated that the decontamination of the crates and trucks was insufficient in several abattoirs commissioned for duck culling during the 2016–2017 HPAI epizootic in France (Huneau-Salaün et al., 2020). This insufficient disinfection of trucks may have contributed to AI dissemination. The preventive culling campaign in January and February 2021 was an opportunity to assess whether the cleaning and disinfection protocols for crates and trucks had improved based on the experience gained from the campaign in 2016–2017. In addition, the previous study relied on environmental sampling coupled with avian influenza virus (AIV) genome detection by real-time reverse-transcription polymerase chain reaction (**rRT**-**PCR**) to evaluate cleaning and disinfection. This protocol has been found to be useful to monitor the effectiveness of control measures against AI (Hood et al., 2019), but it is not commonly used by abattoirs to evaluate cleaning and disinfection of crates and trucks. According to a survey in France in 2019 (ANSES, 2019), abattoirs use visual inspection of surface cleanliness and microbiologic controls such as counts of coliforms on surfaces after decontamination. For coliform enumeration, direct impregnation on culture plates or swabbing are both used for sampling. There is a knowledge gap concerning the correlation between the results obtained by direct detection of AIV genome from surface samples and the results of visual inspection and microbiologic counts on the same surfaces. If correlations were to be identified, routine controls, as currently carried out by the abattoirs, would also be of interest for assessing the risk of AI being spread via crates and trucks. The present study aimed 1) to evaluate cleaning and disinfection of trucks and crates used for the transport of ducks for preventive culling, and 2) to assess correlations between results of methods used to assess crate decontamination by detection of AIV genome, visual inspection of cleanliness, and coliform counts on surfaces.

MATERIALS AND METHODS

Data Collection

Sampling took place in 4 abattoirs (A to D) and at the temporary platform (E) from January 18 to January 20, 2021 by 2 teams of 2 ANSES investigators who collected data on local cleaning and disinfection $(\mathbf{C\&D})$ procedures. The C&D procedures were those applied by the abattoir in charge of crate and vehicle decontamination.

Sampling for Avian Influenza Virus Detection

One to 2 trucks by abattoir were sampled for AIV genome detection, according to the sampling scheme shown in Table 1. Each truck was sampled twice, once before C&D and once after. Sampling of truck surfaces was carried out using a hand fabric swab (moist swab No. 4130, Sodibox, Nevez, France), rubbed on 1 linear meter. Crates were sampled with dry stick-swabs (150c, Murrieta, CA) applied to the corners and slots between crate faces. The same crates could not be sampled before C&D and after C&D, but all crate shipments were tested after unloading from the truck (before C&D) and before reloading on the truck (after C&D). Samples were also taken from the abattoir environment (Table 2). Air sampling (2-4 per abattoir) was performed in the duck shackling cabinet and in the areas for crate and truck cleaning. Air samples were collected using a cyclonebased bioaerosol sampler, Coriolis μ microbial air sampler (Bertin Technologies, Montigny-le-Bretonneux, France): 300 L/min, 10 min/sample, in 10 to 12 mL of 0.005% Triton X-100 solution (Sigma-Aldrich, Saint-Quentin-Fallavier, France) prepared in demineralized water and placed into a sterile sampling cone (Scoizec et al., 2018). For sampling, the investigators wore single-use protective clothing, safety boots, gloves, safety goggles, and a disposable respirator mask with a valve. They wore an extra pair of gloves for sampling and changed them between 2 samples. All samples

Table 2. Number of samples (boot swabs) taken for avian influenza virus genome detection in the environment of the abattoirs (France, 2021).

	Before	e C&D	After C&D			
Site	Area for crate unloading	Area for truck cleaning	Area for crate loading	Area for crate washing		
A	2	1	2	2		
В	2	2	2	2		
С	2	2	2	2		
D	1	1	1	1		
E	1	1	1	1		
Total	8	7	8	8		

(swabs, boot swabs, and air samples) were stored at 4°C until the analysis within 10 d of sampling.

Avian Influenza Virus Detection

Detection of the AIV genome was carried out by rRT-PCR for type A influenza virus, according to the official method (Spackman et al., 2002). Briefly, stick-swabs were diluted in 1 mL of MEM medium supplemented with penicillin and streptomycin. Hand swabs and boot swabs were diluted in 70 mL. RNA extraction were performed from 100 μ L of MEM medium using NucleoMag-VET kit (Macherey-Nagel, Dueren, Germany). Then, 2 μL of RNA extract were tested by rRT-PCR targeting the matrix gene (M gene) of type A influenza using an ADIAVET AIV REAL TIME kit (Bio-X Diagnostics SA, Rochefort, Belgium). If no detection curve was obtained, the RNA extract was retested pure and diluted to 1/10 to test for the presence of PCR inhibitors, according to the manufacturer's specifications. Each run included positive, negative, and internal controls.

The AI status of the slaughtered flocks was established by sampling 20 to 60 ducks per flock using cloacal swabs before transport to or at the abattoir. AI diagnosis was carried out according to the official manual of diagnostics (2006/437/EC), but based on cloacal sampling only.

Visual Inspection and Coliform Counts

Cleanliness of the crates was assessed on 10 crates by shipment after C&D. A 3-score scale was used to describe the cleanliness: clean (no trace of manure or feathers), moderately clean (not completely clean, some traces of manure), and poor (marked soiling by manure and/or feathers). Two methods were used for bacterial sampling: contact place placed on the floor of the crate (1 per crate) and a hand swab rubbed on half of the crate floor (1 per crate). Count plates (ATL coliforms, Laboratoires Humeau, La Chapelle-sur-Erdre, France) contained a violet red bile glucose agar and a disinfectant neutralizer. Plates were incubated for 48 h at 37°C. Enumeration of thermotolerant coliforms from boot swabs was carried out according to standard NF V08-060 (AFNOR, 2009). Results were expressed as the number of colony-forming units (\mathbf{CFU}) per cm² of sampled surface. The detection limits were 0.04 CFU/cm^2 for the count plate method and 0.1 CFU/cm^2 for the swabbing method. The results could not be read over 4 CFU/cm^2 for the plate count method and over 160 CFU/cm^2 for the swabbing method. The same crates were assessed for AIV genome detection, visual inspection, and coliform counts after C&D.

RESULTS

Organization of Crate and Truck Flows

Abattoir ground and floor plans were collected to visualize movement of the trucks within the abattoirs

(Supplementary material 2). In abattoirs A to D, trucks first entered the unloading area to unload crates and then went to the cleaning area. The cleaning area consisted of a concrete platform equipped with cleaning material and a wastewater collection system. After decontamination, trucks went to the loading area to pick up clean crates. In the 4 abattoirs, there was oneway movement from the most soiled areas to the clean ones. However, trucks entered and exited through a single gate in abattoirs A and C. Similarly, the dock for unloading crates with ducks was next to the dock for loading clean crates onto the trucks in abattoirs B, C, and D. The partition wall between both areas was not airtight and airflow was observed from the unloading and shackling areas to the loading area. Regarding crates, they were placed on the conveyor of the washer device after bird unloading. The crate washing room was separated from the bird shackling cabinet by a partition wall, but again, the wall was not airtight. Airflow charged with feathers was observed from the shackling cabinet to the washing room and to the clean crate storeroom. In abattoir E, there was no one-way movement plan. The trucks circulated back and forth on a single road to go to the unloading dock, to the cleaning area, and to the loading dock. The storage area for clean crates (a truck container) was next to the gaseous euthanasia containers and next to the rendering containers.

Protocols for Cleaning and Disinfection

The cleaning and disinfection protocols for trucks and crates are shown in Table 3. Cleaning and disinfection of the trucks was carried out by the truck driver with the protocol and the material provided by the abattoir. Abattoir C was an exception, where the truck was decontaminated by the slaughterhouse staff. In all the abattoirs, the driver was responsible for cleaning the cabin. The abattoir provided certain products, but gave no specific instructions for their use. In fact, only the foot mat was systematically cleaned and disinfected by the drivers. All the protocols for C&D of trucks included treatment with a detergent solution, a high-pressure washing step, and a disinfection step with a virucide product (2 disinfections in abattoir B). More specifically, all abattoirs included treatment with a glutaraldehvde and quaternary ammonia solution; the disinfectant products used in 4 abattoirs were approved against H5N1 avian influenza virus. The manufacturer's recommendations for detergent and disinfectant application were respected in terms of dilution and application times at the 5 premises. Regarding crates, all protocols included a soaking step with a detergent solution, a high-pressure washing step, and one (abattoirs A, D, E) or 2 (B, C) disinfection steps with a virucide solution. For crate disinfection, the immersion method (3 abattoirs) was as frequent as the spraying method (3 abattoirs). Uses of detergent and disinfectant products were again compliant with the manufacturer's recommendations for virus elimination. After C&D, crates were

duck fa	rms against AI (Fra	nce, 2021).	-		0	
Site		А	В	С	D	Е
Truck	Arrival	Wheels are disin- fected by spraying	Wheel bath with disinfectant	Wheel bath with disinfectant	No disinfection	Wheels are disin- fected by spraying
	Washing	Soaking with foam- ing detergent High-pressure washing with water at 45°C	Soaking with foam- ing detergent High-pressure washing with water at 60°C	Soaking with foam- ing detergent High-pressure washing with water at 55°C	Soaking with foam- ing detergent High-pressure washing with water at 50°C	Spraying with deter- gent High-pressure washing with water at room temperature
	Disinfection	Disinfection by spraving	Two disinfections by spraving	Disinfection by spraving	Disinfection by spraving	Disinfection by spraving
	Departure	Wheels are disin- fected by spraying	Wheels are disin- fected by spraying	Wheel bath with disinfectant	No disinfection	Wheels are disin- fected by spraying
Crate	Soaking - detergent	Washing tunnel: soaking with deter- gent solution, high-pressure washing and rins- ing with water at 55°C	Washing tunnel: soaking with deter- gent solution, high-pressure washing and rins- ing with water at 60°C Spraying with water at 80°C	Washing tunnel: soaking with deter- gent solution, high-pressure washing and rins- ing with water at 60°C In case of non-com- pliant washing ¹ : high-pressure	Washing tunnel: soaking with deter- gent solution, high-pressure washing and rins- ing with water at 60°C	Soaking with deter- gent solution High-pressure washing with water at room tem- perature In case of non-com- pliant washing ¹ : high-pressure washing

First disinfection by

by immersion

spraying Second disinfection washing

First disinfection by

spraying Second

disinfection by

immersion

Table 3. Cleaning and disinfection protocols for transport crates and trucks used in 5 abattoirs during the preventive depopulation of

¹Visual evidence of organic residues.

Disinfection

loaded onto the clean truck immediately. There was no drying period.

Disinfection by

immersion

Detection of Avian Influenza Virus Genome Before and After Cleaning and Disinfection

Before C&D, 3 quarters of the sampling crates (59/79)were positive for AIV genome (Table 4). Seven out of 8 shipments of crates were positive for AIV genome (Table 5) because they transported ducks detected positive for AI on the farm. On the contrary, the duck batch transported in Truck 7 (abattoir A) tested negative for

AI before transport and no crates of that shipment were detected positive for AIV genome before C&D. All trucks were positive for AIV genome on the outside, on the wheels, or in the cabin except Truck 7, which transported ducks negative for AI. The outside of the truck (truck bed and rocker panels) and the cabin were more frequently positive for AIV genome than the wheels. Importantly, wheels and mudguards were disinfected at the entrance in 4 abattoirs. After C&D, 29% of the crates (23/80) were positive for AIV genome vs. 75%before C&D (chi-square test, P < 0.001). There were positive crates in 6 shipments of cages out of 8, including the shipment of Truck 7 (2/10 positive for AIV genome);

Spraving disinfection

Spraving disinfection

Table 4. Detection of the avian influenza virus genome by rRT-PCR based on environmental sampling before and after cleaning and disinfection in 5 duck abattoirs in France in 2021.

Before C&D									
		${ m M}\ { m gene}\ { m rRT-PCR}\ { m result}$	Ct M gene rRT-PCR value						
	Not detected	Detected	% detection	Median	Min-max				
Cabin	3	5	62	35.3	34.9-37.2				
Outside of truck	1	7	87	35.1	29.3 - 39.9				
Wheel	5	3	38	37.1	35.4 - 37.1				
Crate	20	59	75	32.1	26.2 - 37.4				
Total	29	74	72	32.8	26.2 - 39.9				

		After	C&D		
		${ m M}\ { m gene}\ { m rRT-PCR}\ { m result}$	Ct M gene rRT-PCR value		
	Not detected	Detected	% detection	Median	Min-max
Cabin	5	3	38	37.1	35.3 - 39.6
Outside of truck	7	2	22	34.2	32.3 - 36.2
Wheel	7	1	12	36.6	
Crate	57	23	29	36.4	31.1 - 38.2
Total	76	29	28	36.4	31.1 - 39.6

Table 5. Detection of the avian influenza virus genome based on environmental sampling before and after cleaning and disinfection in 5 abattoirs in France in 2021.

Site Truck Sample Before After А 0/92/107 crate 8* cabin 1/1 $\dot{0}/1$ outside¹ of truck 1/10/110/102/10crate В 5* cabin 1/10/1outside of truck 1/11/1crate 10/102/106* cabin 1/10/1outside of truck 0/11/1wheel 1/10/110/108/10crate С 1* cabin 0/11/1outside of truck 1/10/1wheel 1/10/19/10 0/10crate 2* outside of truck 0/11/10/10crate 1/10D 3* cabin 1/11/1outside of truck 1/10/1wheel 1/11/1crate 9/10 2/10 Е 4* cabin 1/11/1outside of truck 1/11/29/107/10 crate

¹Outside of the truck: truck bed and rocker panels.

 $^{*}\mathrm{Trucks}$ transporting HPH5 AIV-positive ducks. Results in bold are positive.

no AIV genome trace was detected on this shipment before C&D. By contrast, no residual contamination was observed for the 2 shipments of crates assessed in Abattoir C. After C&D, 31% of the samples taken on truck surfaces (6/19) were positive for AIV genome. The frequency of positive samples was higher in the cabins, which were not disinfected, than on the outside and the wheels.

Table 6. Results of cleanliness visual inspection, coliform counts, and detection of avian influenza virus genome on crates for duck transport after cleaning and disinfection in 5 abattoirs in France in 2021.

		M gene rRT-PCR result					
Method	Result	Not detected	Detected	% detected			
Cleanliness	Clean	48	19	28			
	Moderate	5	4	44			
	Poor	4		0			
Swabbing	<detection limit<="" td=""><td>54</td><td>20</td><td>27</td></detection>	54	20	27			
~	$<10 \mathrm{CFU}/\mathrm{cm}^2$	1		0			
	$<100 \text{ CFU}/\text{cm}^2$	1	3	75			
	Not countable	1		0			
Plate count	<detection limit<="" td=""><td>55</td><td>21</td><td>28</td></detection>	55	21	28			
	$<1 \mathrm{CFU/cm}^2$		1	100			
	$<10 \mathrm{CFU/cm^2}$	1		0			
	Not countable	1	1	50			

Cleanliness Assessment and Microbial Counts

Eighty crates were visually assessed for cleanliness after C&D. Sixty-seven crates out of 80 (84%) were considered clean, while moderate cleanliness (9 crates, 11%) and poor cleanliness (4 crates, 5%) were sometimes observed in Abattoirs A and E. Regarding microbiologic assessments, 92% (74/80) of the coliform counts by swabbing were below the detection limit (Figure 1). Similarly, 95% (76/80) of the count plates showed no coliform colony. No residual coliform contamination (by swabbing and by count plate) was then detected for 91% (73/80) of the crates. No correlations were observed between the results for AIV genome detection and the results for visual inspection or coliform counts (Table 6). As an example, 28% of the crates that were considered clean were positive for AIV genome.



Figure 1. Results for coliform counts on crates for duck transport after cleaning and disinfection by sampling method (plate count and hand swabbing) in 5 abattoirs in France in 2021. Sites are identified from A to E and trucks from 1 to 8.



Figure 2. Detection of the avian influenza virus genome, based on sampling by boot swabs, on different floor surfaces in 5 abattoirs in France in 2021.

Detection of Avian Influenza Virus Genome in the Environment

AIV genome was detected in 22 out of 31 samples (71%) taken by boot swabs on the truck traffic areas and on the floor of the crate washing room (Figure 2). Seven out of 8 samples on crate unloading docks and 4 out of 7 samples taken on truck washing areas were positive prior to the arrival of the vehicle. Fifteen air samples were collected. AIV genome was detected in all samples: in the unloading crate area and the shackling cabinets (10/10), in the crate washing room (2/2), in the truck washing area (2/2), and near the rendering container (1/1).

DISCUSSION

Changes in Cleaning and Disinfection Protocols

The study carried out in 2017 had highlighted organizational difficulties in the decontamination of vehicles and animal transport crates (Huneau-Salaün et al., 2020). In particular, the separation of dirty and clean areas was a problem. An improvement was observed in the current study, but 2 abattoirs had a single gate (and a single wheel bath) for entry and exit of the trucks, leading to a risk of cross-contamination. In addition, significant airflows were still present between dirty and clean areas, despite the presence of separation panels. AIV genome was detected in air samples taken in the unloading area in all the abattoirs. Therefore, airflow may cause recontamination of the crates after disinfection as the detection of AIV genome in the air of the crate washing and storage room in 2 abattoirs (A & B) strongly suggests this. Moreover, the floor of the washing and storage rooms for crates and the floor of the decontamination area for trucks were frequently positive for AIV genome. These surfaces were disinfected at the end of the working day only. They should be disinfected regularly throughout the working day to limit the risk of cross-contamination. As an example, cross-contamination could not be ruled out in the case of the crate shipment of Truck 7, which was negative for AIV genome before C&D, but positive after C&D. The C&D organization was even more difficult for the depopulation platform (E), which was a temporary facility. The storage area for clean crates was next to the rendering containers and the gas euthanasia containers. Feather fallouts on clean crates were observed during sampling, leading to likely contamination of the crates. This may explain poor results for C&D at site E. These observations suggest the need to identify in advance in AI contingency plans sites large enough to accommodate temporary platforms with a clear separation between the dirty and clean areas.

Crate decontamination protocols varied little between abattoirs. Compared to 2017, the decontamination protocols all included spraying of a detergent solution. Use of hot water was systematic, whereas it was only used in 2 out of 4 abattoirs in 2017. Choices of disinfectant and their application conditions were appropriate for AI elimination, contrary to previous observations in one abattoir in 2017. These parameters may sometimes be imperfectly applied in field conditions (Kim et al., 2020), but this does not seem to be the case in the present study. Despite these improvements in C&D protocols, the results after decontamination did not show any improvement in performance with 29% (23/80) of the crates testing positive for AIV genome in 2021 compared to 21% in 2017 (43/200 for 13 shipments of crates tested). Abattoir C was the only one where no contamination was detected on the crates after disinfection, as in 2017. This abattoir had a very long crate washing line, including a final disinfection step by immersion, and very strict separation between dirty and clean areas, which were on opposite sides of the building. The vehicle decontamination protocols were also more robust in 2021 than in 2017, with the addition of foaming treatment with a detergent product during washing. However, 2 trucks out of 8 had residual contamination on the outside in 2021, compared to 4 out of 13 in 2017. In addition, cabins remained a risk point, with frequent positive samples after decontamination steps because they were not decontaminated directly, as in 2017.

Methods for Cleaning and Disinfection Quality Assessment

One of the aims of the study was to validate the use of visual inspection and bacterial count methods to assess the quality of C&D against AIV on crates and trucks. The foie-gras production sector in France adopted a good practice guide for hygiene in duck transport in 2016 (CIFOG, 2016). On the basis of this guide, crate decontamination is considered satisfactory when the coliform count is lower than 2 CFU/cm^2 (sampling with count plates). This was the case for 96% of the crates sampled in the study. However, results for AIV genome detection after decontamination were not as good as results for visual inspection and bacterial count. In addition, no correlation was observed between the AIV genome detection results and results of cleanliness inspection and microbiological counts. Regarding visual inspection, it is common to observe uncorrelated results between cleanliness and microbiological counts (Allen et al., 2008; Atterbury et al., 2020). Visual inspection is a prerequisite before microbiological testing; the latter should be performed on visually clean surfaces only. As a result, visual cleanliness assessments are needed for complete evaluation of C&D, but this is not an indicator of the effectiveness of decontamination with regard to the AI risk. The lack of correlation between the results for bacteriological and genomic viral controls might be linked to the choice of surfaces sampled on the crates. The hand swabs and contact plates for bacterial counts were applied on the floor of the crate, whereas the stick-swabs for AIV genome detection were taken from the corners and slots between crate faces. Sampling from areas that are difficult to access, such as corners and slots, is of interest to detect the presence of the AIV genome (Indriani et al., 2010), but this technique is not suitable for bacterial counts. Therefore, coliform enumeration is not a relevant indicator of residual AI genome contamination. It is not a suitable substitute method for environmental sampling coupled with detection of AIV genome by rRT-PCR. RT-PCR is not currently used at the abattoir in France. The main limit of this method is that a positive result denotes the presence of AIV genome but does not inform on virus ability to infect poultry. Only virus isolation and titration

directly inform on the risk of AI transmission. However, environmental sampling coupled with detection of AIV genome by rRT-PCR is widely used, both for the evaluation of AI control measures (Kang et al., 2015; Chowdhury et al., 2020) and for disease surveillance (Hood et al., 2019). This method is easy to apply and remains affordable (around 30 euros/sample), despite its cost being higher than bacteriologic methods (around 5 euros/sample for the plate count method and 13 to 15 euros/sample for enumeration on swabs). Therefore, the abattoirs should be encouraged to use this method to assess the efficacy of decontamination of crates and trucks during AI epizootics in order to limit the risk of AI spread by transport activities.

CONCLUSION

Cleaning and disinfection procedures for decontamination of trucks and crates were significantly improved since the last AI epizootic in 2017 in France. The results of visual inspections and of microbiological counts showed that cleaning and disinfection were compliant with hygiene standards commonly applied in the abattoirs. Nevertheless, residual traces of AIV genome were frequently observed on crates and trucks, due to cross-contaminations likely to occur at several points in the C&D process. Therefore, using an adequate method to monitor the efficacy of decontamination procedures, involving environmental sampling coupled with AIV genome detection, is recommended to further improve C&D practices.

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DISCLOSURES

The authors have no conflicts of interest to disclose.

SUPPLEMENTARY MATERIALS

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j. psj.2021.101569.

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