IMMUNOLOGY, HEALTH AND DISEASE

Cleaning and disinfection of crates and trucks used for duck transport: field observations during the H5N8 avian influenza outbreaks in France in 2017

Adeline Huneau-Salaün,[1] Axelle Scoizec, Rodolphe Thomas and Sophie Le Bouquin

ANSES – Laboratoire de Ploufragan-Plouzané-Niort, Ploufragan, France

ABSTRACT Transport of infected birds is thought to play a key role in the spread of avian influenza (**AI**) on poultry farms during epizootic outbreaks. Ensuring efficient cleaning and disinfection (**C&D**) of equipment used for transport is needed to prevent the spread of AI. This study aimed to evaluate the efficacy against the AI virus of C&D protocols applied on trucks and crates used for the transport of ducks during the H5N8 AI outbreaks in France in 2017. In 3 abattoirs, 16 transport vehicles and their crates were sampled by swabbing to detect the influenza type A genome by real-time reverse-transcription polymerase chain reaction. Vehicles were tested before and after decontamination, which was carried out in accordance with the abattoirs' protocols. A total of 86 samples out of 299 collected before C&D were positive for AI (29%); 7 trucks out of 16 transported crates detected positive for AI. After C&D, the AI genome was detected in 56 samples out of 308 (18%). Ten trucks were loaded with a shipment of AI-positive crates. Eight vehicles were detected positive in the cabin, on the truck bed, and/or on the wheels. Despite reinforcement of C&D, the efficacy of decontamination was variable among slaughterhouses. The efficacy seemed to depend on the initial contamination load, C&D protocols, and how the protocol is implemented. Breaks in biosecurity measures led to frequent contamination of trucks after C&D. Observational studies during animal health crises are of interest to analyze practices in emergency conditions and to put forward measures aimed at increased preparedness.

Key words: influenza, transport, duck, cleaning, disinfection

2020 Poultry Science 99:2931–2936
https://doi.org/10.1016/j.psj.2019.10.015

INTRODUCTION

From November 2016 to April 2017, France faced the largest outbreak of HP avian influenza (**AI**) in commercial poultry farms that had occurred in the European Union since the 2000s. In total, 484 outbreaks were detected, caused by HP H5 AI virus A/Goose/Guangdong/1/96 clade 2.3.4.4. About 3 quarters of the outbreaks occurred on farms rearing Mulard ducks. Most of the infected flocks did not show clinical signs, and all the infected farms were located in the southern part of France. As a consequence, preventive stamping out of all duck farms in this area was imposed from April to May 2017. All the flocks of ducks reared for foie gras production were slaughtered and destroyed in local abattoirs that were requisitioned for this purpose. The birds were transported to the abattoir in plastic crates that were loaded onto a truck; the crates and trucks were to be cleaned and disinfected after transport, as required by the European Council Directive 2005/94/EC on AI control measures.

However, the efficacy of cleaning and disinfection (**C&D**) procedures as applied in the abattoirs remained undocumented for crates and trucks used for the disposal of AI-infected birds. Effective C&D of crates and trucks remains difficult for most poultry operators (Burton et al., 2005; Northcutt et al., 2006; Musavian et al., 2015). As an example, series of trials carried out in several slaughterhouses in the UK showed that there was little difference in bacterial load of crate surface before and after decontamination (Burton et al., 2005). During AI outbreaks, effectiveness of disinfection implies that the residual load of infectious AI particles on the treated surface is lower than the minimal infectious dose. Such references do not exist for the indirect transmission of AI via a soiled surface. In addition, virus isolation and titration on embryonating chicken eggs needed to quantify the residual viral load are labor and time-consuming; these methods can hardly be used for assessing C&D procedures during outbreaks situations. An alternative strategy is to use environmental sampling

Received June 11, 2019.
Accepted October 1, 2019.

[1]Corresponding author: adeline.huneau@anses.fr

coupled with AI genome detection by real-time reverse-transcription polymerase chain reaction (**rRT-PCR**). A positive result denotes the presence of AI genome but does not inform us about virus viability, capacity of infection, or pathogenicity. Nevertheless, this protocol showed its interest for monitoring the effectiveness of control measures taken in live poultry markets to limit the H7N9 epidemic wave in Guangdong, China, in 2013 (Kang et al., 2015); valid comparisons between predecontamination and postdecontamination detection of AI genome could be made. The present study focused on the assessment of C&D of trucks and crates used for the preventive culling of duck flocks in France in 2017; the assessment is based on an environmental sampling monitoring protocol for the detection of AI genome.

MATERIALS AND METHODS

Data Collection

This observational study aimed to compare frequencies of AI genome detection on duck transport crates and trucks before and after decontamination. Six visits for sampling were carried out in 3 duck abattoirs from January to March 2017. The C&D protocols tested were those applied by the abattoir in charge of the decontamination of the vehicle. Information about the C&D protocols was collected in a standardized questionnaire filled in during the visits at the abattoir. A trained investigator conducted all the visits. For sampling, he wore single-use protective clothing, safety boots, gloves, goggles and a disposable respirator mask with a valve. He put on an extra pair of gloves for sampling and changed it between 2 samples.

Sampling

Two to four trucks were sampled during a visit by a single operator, according to the sampling scheme in Table 1. Sampling was carried out during decontamination operations without changing the operators' work rhythm: the number of samples was therefore limited so as not to slow down the work. The sampling protocol was more focused on the crates, which were the most likely surfaces to be contaminated during the transport of infected ducks. If not all samples could be made because of time constraint, priority was given to sampling crates over sampling trucks or C&D areas. Each truck was sampled twice, once before C&D and once after C&D. Truck surfaces were sampled with a dry fabric swab (swab N°4130, Sodibox, Nevez, France), rubbed on 1 linear meter. Regarding crates, the same crate could not be sampled before and after C&D, but each batch of crates, that is a shipment of crates unloaded from and reloaded on a given truck, was checked before and after C&D. Crates were sampled with one fabric swab rubbed on one half of the crate floor, with 2 dry stick swabs (150c, Murrieta, CA 92562) applied to the corners and slots between crate faces, which are the parts least accessible to cleaning. Up to 5 crates by shipment were sampled before and after C&D. Areas dedicated to truck decontamination were sampled using boot swabs (swab N°4136, Sodibox, Nevez, France) by walking on the concrete floor for 3 min. The 2 boot swabs were pooled into a single sample for analysis. Samples were stored at 4°C and transported to the laboratory within 4 h.

AI Detection

Detection of the AI genome was carried out by rRT-PCR for type A influenza virus, in accordance with the official method (Spackman et al., 2002). In brief, swabs were diluted in 15 ml of Glasgow medium (Merck, Lyon, France) and shaken manually. An RNeasy Mini Kit© (Qiagen GmbH, Courtabeouf, France) was used for RNA extraction from 200 µl of broth. Two microliters of RNA extract from the 50 µl obtained from purification were tested by rRT-PCR targeting the matrix gene (M gene) of type A influenza viruses (Spackman et al., 2002; Cherbonnel et al., 2013). Each run included positive, negative, and internal controls.

The visits at the abattoirs were planned according to logistic constraints without knowing the AI status of flocks to be slaughtered on the day of the visit. The AI status of the slaughtered flocks was established by

Table 1. Number of samples (swabs and stick swabs) taken for AI genome detection on transport crates and trucks (France, 2017).

			Before C&D						After C&D					
				Truck						Truck				
Abattoir	Date	Number of trucks	Crate	Ext[1]	Wheel	Cabin[2]	C&D area[3]	Tot.	Crate	Ext	Wheel	Cabin	C&D area	Tot.
A	24/01	4	60[4]	3	3	9	4	79	60	4	4	12	6	86
A	27/01	2	30	2	2	6	2	42	30	2	2	6	2	42
B	26/01	4	60	4	4	12	1	81	60	4	4	12	2	82
B	30/01	2	30	3	2	6	1	42	30	2	2	6	2	42
C	17/03	2	20	2	2	2	1	27	20	2	2	2	2	28
C	20/03	2	20	2	2	2	2	28	20	2	2	2	2	28
Total		16	220	16	15	37	11	299	220	16	16	40	16	308

Abbreviation: C&D, cleaning and disinfection.
[1]Truck bed and rocker panels.
[2]Steering wheel, gear lever, and handles.
[3]Areas for crate unloading and truck cleaning and disinfection.
[4]Three samples were taken by crate: 1 swab and 2 stick swabs.

Table 2. Cleaning and disinfection protocols for transport crates and trucks used in 4 abattoirs during the preventive stamping out of duck farms against AI (France, 2017).

Abattoir	A	B	C
Truck	When arriving: wheels are disinfected by spraying High-pressure washing Disinfection by spraying When leaving: wheels are disinfected by spraying	When arriving: wheels are disinfected by spraying High-pressure washing Disinfection by spraying When leaving: wheels are disinfected by spraying	When arriving: automatic wheel washing system with a disinfectant solution High-pressure washing with water at 60°C Foam disinfection When leaving: automatic wheel washing system with a disinfectant solution
Cabin	Sprayer filled with a disinfectant solution is provided to the driver	No C&D	No C&D
Crate			
Soaking—detergent	Washing tunnel: soaking with detergent solution, low-pressure washing,[1] and rinsing In case of noncompliant washing[2]: high-pressure washing	Low-pressure washing Soaking in water at 80°C for 5 s.	Low-pressure soaking with detergent solution at 60°C Soaking in a detergent solution at 60°C for 20 s.
Disinfection	Spraying disinfection	Spraying disinfection	Soaking in a disinfectant solution at 20°C for 20 s. In case of noncompliant washing: low-pressure washing and spraying disinfection

Abbreviation: C&D, cleaning and disinfection.
[1]Lines of low-pressure nozzles.
[2]When the operator carrying the cleaning considers the quality of the washing unsatisfactory (organic material residues).

sampling 20 ducks per flock using cloacal swabs during antemortem inspection. The sampling was performed by the veterinary officers at the abattoirs. AI diagnosis was carried out in accordance with the official manual of diagnostics (2006/437/EC) but based on cloacal sampling only.

RESULTS AND DISCUSSION

Protocols for C&D

Protocols for C&D are shown in Table 2. The protocols for C&D of crates and trucks were different among abattoirs depending on the equipment available for cleaning. The choice of equipment for cleaning was mainly driven by the area available in the facility dedicated to crate decontamination. Disinfection products used in abattoirs A and C were commercial solutions of quaternary ammonium compounds (**QAC**) with glutaraldehyde, recommended for AI virus elimination; the concentration recommended by the manufacturer for AI disinfection was applied. The combination of QAC and glutaraldehyde has been proven to be effective against both LP AI H7N2 virus (Davidson et al., 1999) and HP H5N1 virus (Wanaratana et al., 2010). In abattoir B, a commercial solution of QAC was used at the dilution recommended by the manufacturer for AI elimination at 20°C. However, the disinfection was carried out in a cold atmosphere, with the ambient temperature below 10°C (on an open-air concrete area). No minimum contact time was defined in the protocols for disinfection, except for soaking disinfections. In that last case, the crates were totally submerged in a soaking tub for 20 s. Whatever the method used for disinfection (spraying or soaking), the disinfectant solution was applied on wet crates and was left to dry naturally.

Detection of AI Genome Before C&D

A total of 86 samples out of 299 (28.8%) obtained before C&D were positive for AI (Table 3, Figure 1). About one-third of the samples taken on crates were positive (74/220, 33.6%), and 10 shipments of crates out of 16 (62.5%) were positive before C&D (Table 4). Seven trucks were also positive for AI on the truck bed (4 cases), on the wheels (1 case), and/or in the driving cabin (3 cases). AI testing of ducks showed that flocks positive for HPAI H5N8 were slaughtered during the first visit at abattoirs A (4/4 positive flocks), B (2/4 positive flocks), and C (1/2 positive flocks). As a consequence, crates and

Table 3. Detection of the influenza virus genome by rRT-PCR based on environmental sampling before and after cleaning and disinfection (C&D) in 3 duck abattoirs in France in 2017.

Before C&D			
	M Gene rRT-PCR result		
	Not detected	Detected	% Detection
Cabin	34	3	8
Ext. truck	16	4	20
Wheel	10	1	9
Crate	146	74	34
C&D area	7	4	36
Total	213	86	29
After C&D			
	M gene rRT-PCR result		
	Not detected	Detected	% Detection
Cabin	34	6	15
Ext. truck	17	3	15
Wheel	11	1	8
Crate	177	43	19
C&D area	13	3	19
Total	252	56	18

Abbreviations: C&D, cleaning and disinfection; rRT-PCR, real-time reverse-transcription polymerase chain reaction.

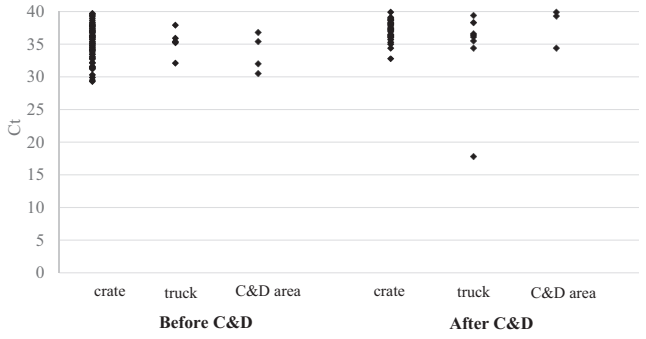

Figure 1. Detection of the influenza virus genome by rRT-PCR on duck transport crates and trucks (France, 2017). Abbreviations: C&D, cleaning and disinfection; rRT-PCR, real-time reverse-transcription polymerase chain reaction.

trucks that transported these animals were all positive for influenza genome before C&D. No other infection by AI virus was detected on flocks positive for HPAI H5N8 virus. Flocks processed at the other visits (27/01, 30/01, and 20/03) were not detected to be AI positive (including non–H5-H7 virus). On the days when noninfected birds were processed, the AI genome was not detected on trucks and crates before C&D but was found on C&D areas before C&D in abattoir A (27/01) and in abattoir B (30/01).

Detection of AI Genome After C&D

After C&D, the AI genome was detected in 56 samples out of 308 (18%). Positive crates after C&D were frequently observed during the first visits at abattoirs A and B in relation with a high frequency of contaminated crates before C&D. C&D protocols for crates in these abattoirs, based on disinfection by spraying, appeared to be insufficient to reduce crate contamination. On the contrary, no residual detection of AI genome was observed in abattoir C on the first visit (17/03) despite that the crates were frequently positive before C&D. The C&D protocol in this abattoir relied on 2 cleaning steps with detergent and hot water (low-pressure cleaning and soaking) and 1 disinfection step by soaking. Surprisingly, the AI genome was detected on 2 batches of crates after C&D in abattoir B on the second day of visit (30/01), while no contamination was found before C&D. Cross contamination may have occurred owing to insufficient C&D of the equipment and area after treating a previous batch of contaminated crates. Areas dedicated to crate cleaning were generally small, making it impossible to implement sanitary barriers between the area for unloading dirty crates and the 1 for loading disinfected crates onto the truck.

Seven trucks out of 16 were positive for the AI genome after C&D in the driving cabin (4 cases) and/or on the wheels and truck bed (4 cases). No contamination was detected before C&D for 3 of these trucks. In the abattoirs, the surroundings of the main building were not large enough to organize forward traffic of the trucks from the reception area to the cleaning area and the loading area. Crossing between contaminated and disinfected trucks was thus frequent, in contradiction with common biosecurity measures. In addition, C&D areas were already contaminated before truck cleaning on 3 occasions. Residual contamination in some driving cabins was expected as no C&D of cabins was performed. Drivers regularly walked on C&D areas without changing boots or wearing boot clothes, although these areas were frequently detected positive for AI. Contamination of the cabin floor may thus occur. Similarly, wheels of the trucks could be contaminated by passing over these areas. Furthermore, drivers took part in crate unloading without changing gloves before driving the truck, leading to contamination of the steering wheel, gear lever, and handles. Biosecurity measures for unloading crates and C&D measures for the truck cabin, for the C&D area, and for the traffic lanes are therefore needed to avoid cross contamination.

Importance and Limitations

This study has several limitations linked to the difficulty of carrying out sampling during a period of animal health crisis. The number of samples was limited to ensure that sampling did not slow the abattoir's activity. Similarly, the processing capacities of laboratories must be reserved primarily for diagnosis. Some tests, such as AI virus isolation, could hardly be performed from environmental samples taken in this context. The choice for C&D evaluation was therefore made for a simple and inexpensive method (swabbing and M gene rRT-PCR), even if it does not provide complete information on the viability of the residual viruses and on the actual health risk involved. Nevertheless, observational studies during animal health crises are of interest to analyze practices in emergency conditions and to propose measures to increase preparedness. Our findings emphasized that inadequate organization and equipment for C&D result in high levels of residual contamination and likely cross contamination.

Environmental sampling and M gene rRT-PCR were found to be of interest in evaluating C&D efficacy. First, extensive contamination before C&D was observed in association with the transport of AI-infected birds. However, no initial contamination was detected on the crates on the days when only noninfected ducks were processed (27/01, 30/01, and 20/03). Second, some variations in frequency of detection were observed after C&D, indicating differences in the efficacy of the applied protocols. As an example, frequently contaminated crates were treated with different C&D protocols in abattoirs A and C; no residual contamination was observed in abattoir C, while the results were often positive after C&D in abattoir A. Our evaluation protocol made it possible to capture this heterogeneity in C&D results. Third, frequencies of positive samples from crates were similar with the fabric swab and the 2 stick swabs (Table 5).

However, 56% of the positive crates (18/32) were detected by a single type of sampling before C&D: 14 crates were positive based on swabs only, and 4 crates

Table 4. Detection of the influenza virus genome based on environmental sampling before and after cleaning and disinfection (C&D) in 3 duck abattoirs in France in 2017. Results in bold are positive results.

Abattoir	Date	Truck	Sample	Before	After
A	24/01	1[3]	Cabin	n.s.[1]	**3/3**
			Ext[2]. truck	n.s.	**1/1**
			Wheel	n.s.	**1/1**
			Crate	**14/15**	**10/15**
		2[3]	Cabin	**1/3**	0/3
			Ext. truck	0/1	0/1
			Wheel	0/1	**1/1**
			Crate	**9/15**	**6/15**
		3[3]	Cabin	0/3	0/3
			Ext. truck	**1/1**	0/1
			Wheel	0/1	0/1
			Crate	**15/15**	**5/15**
		4[3]	Cabin	0/3	**1/3**
			Ext. truck	0/1	0/1
			Wheel	0/1	0/1
			Crate	**9/15**	**7/15**
		C&D area		**1/4**	**3/6**
	27/01	1	Cabin	0/3	0/3
			Ext. truck	0/1	**1/1**
			Wheel	0/1	0/1
			Crate	0/15	0/15
		2	Cabin	0/3	0/3
			Ext. truck	0/1	0/1
			Wheel	0/1	0/1
			Crate	0/15	0/15
		C&D area		**2/2**	0/2
B	26/01	1[3]	Cabin	**1/3**	0/3
			Ext. truck	0/1	0/1
			Wheel	0/1	0/1
			Crate	**7/15**	**4/15**
		2[3]	Cabin	0/3	0/3
			Ext. truck	**1/1**	**1/1**
			Wheel	0/1	0/1
			Crate	**6/15**	**5/15**
		3	Cabin	**1/3**	**1/3**
			Ext. truck	0/1	0/1
			Wheel	0/1	0/1
			Crate	**1/15**	**2/15**
		4	Cabin	0/3	0/3
			Ext. truck	**1/1**	0/1
			Wheel	**1/1**	0/1
			Crate	**1/15**	**2/15**
		C&D area		**1/1**	0/2
	30/01	1	Cabin	0/3	0/3
			Ext. truck	0/1	0/1
			Wheel	0/1	0/1
			Crate	0/15	**1/15**
		2	Cabin	0/3	0/3
			Ext. truck	0/2	0/1
			Wheel	0/1	0/1
			Crate	0/15	**1/15**
		C&D area		0/1	0/2
C	17/03	1[3]	Cabin	0/1	0/1
			Ext. truck	**1/1**	0/1
			Wheel	0/1	0/1
			Crate	**9/10**	0/10
		2	Cabin	0/1	**1/1**
			Ext. truck	0/1	0/1
			Wheel	0/1	0/1
			Crate	**3/10**	0/10
		C&D area		0/1	0/2
	20/03	1	Cabin	0/1	0/1
			Ext. truck	0/1	0/1
			Wheel	0/1	0/1
			Crate	0/10	0/10
		2	Cabin	0/1	0/1
			Ext. truck	0/1	0/1
			Wheel	0/1	0/1
			Crate	0/10	0/10
		C&D area		0/2	0/2

were detected by fabric swabbing only. After C&D, 16 crates were detected positive based on swabbing only and 6 based on fabric swabbing; 8 crates yielded positive results by both sampling methods. Using both swab samples and fabric swab samples is therefore important to improve the AI detection on the crates. Fabric swabs make it possible to easily sample a very large area for pathogen detection, whereas swabs are more suitable for sampling hard-to-reach surfaces (Galvin et al., 2012). The proposed method of C&D evaluation based on environmental swabbing coupled with M gene rRT-PCR needs to be further validated by cross-validation with other commonly used methods, such as visual cleanliness inspection and monitoring of microbial indicators (Allen et al., 2008). The cross-validation is needed to assess whether classical methods for C&D evaluation may provide information on the AI risk in hazard analysis and critical control points–based systems.

Differences in C&D protocols and in their application have led to heterogeneous results between slaughterhouses. On the crates, no visible residue of organic matter was observed after C&D in general, but the swabs were often soiled after rubbing the surfaces. Crate decontamination seemed to be most effective in slaughterhouse C, which was the only one that applied 2 steps of soaking with detergent and disinfection by soaking. These practices would be interesting to generalize, but they require facilities that are not necessarily available in all slaughterhouses. The requisition of a few slaughterhouses for the slaughter of infected animals allows a better organization of operations and limits the duration of the depopulation process in the affected areas. However, specific means should be provided to these slaughterhouses to enable them to carry out effective decontamination of the equipment. A standard protocol, validated experimentally, should be provided to operators for all elements to be cleaned and disinfected. A preliminary experimental study would make it possible to design an effective C&D protocol for crates and trucks. In experimental conditions, the complete evaluation of the protocol could be carried out, by including virus isolation from samples positive for AI virus genome after C&D. Based on this protocol, material and human needs could be defined in the slaughterhouse requisition plan, increasing the preparedness in case of sanitary crisis.

Although all the abattoirs reinforced their C&D protocols to mitigate the risk of AI spread, C&D efficacy was variable among slaughterhouses. Cleaning and disinfection efficacy seemed to depend on initial contamination load, C&D protocols, and the quality of protocol application. Further improvements in C&D protocols and reinforcement of biosecurity measures at abattoirs are needed to avoid residual contamination of the equipment and cross contamination during the decontamination process. Despite some limitations due to logistic constraints,

[1]Not sampled.
[2]Exterior of the truck: truck bed and rocker panels.
[3]Trucks transporting ducks positive for HPH5 AI virus by virological testing on cloacal swabs.

Table 5. Detection of the influenza virus genome by rRT-PCR on duck transport crates using fabric swabbing or swabbing (France, 2017).

Before C&D					
	M Gene rRT-PCR result			Ct M gene rRT-PCR value	
	Not detected	Detected	% Detection	Median	Range
Fabric swab	55	25	33	35.0	29.3–39.4
Swab	91	49	35	35.8	29.9–39.7
After C&D					
	M gene rRT-PCR result			Ct M gene rRT-PCR value	
	Not detected	Detected	% Detection	Median	Range
Fabric swab	66	14	17	36.7	32.8–38.8
Swab	111	29	21	37.4	34.4–39.9

Abbreviations: C&D, cleaning and disinfection; rRT-PCR, real-time reverse-transcription polymerase chain reaction.

observational studies during animal health crises are valuable for capitalizing on experience and proposing improvements in biosecurity and decontamination procedures.

ACKNOWLEDGMENTS

The authors are grateful to the companies and the company staff who took part in the study, to the *Laboratoire des Landes et des Pyrénées*, to the local veterinary authorities, and to Mathias Delpont from the Veterinary School in Toulouse. The authors would also like to thank the French Ministry of Agriculture for its financial support. The authors have no conflicts of interest to disclose.

REFERENCES

Allen, V. M., C. H. Burton, D. J. Wilkinson, R. T. Whyte, J. A. Harris, M. Howell, and D. B. Tinker. 2008. Evaluation of the performance of different cleaning treatments in reducing microbial contamination of poultry transport crates. Br. Poult. Sci. 49:233–240.

Burton, C. H., R. T. Whyte, V. M. Allen, and D. B. Tinker. 2005. Reducing microbial contamination from poultry transport crates by improved cleanind and disinfection systems based on better water use. Report, Silsoe Research institute, Bristol, UK.

Cherbonnel, M., A. Henry, K. Ogor, and V. Jestin. 2013. Validation of a duplex real-time RT-PCR assay to detect avian influenza virus matrix gene using armored RNA as an internal positive control. In: WVPAC (Ed.), *Book of Abstracts*, 19-23 August 2013, p. 446. Nantes, France.

COUNCIL DIRECTIVE 2005/94/EC of 20 December 2005 on Community measures for the control of avian influenza and repealing Directive 92/40/EEC. Official J. Eur. Union, 14/01/2006 L10.16.

Davison, S., C. E. Benson, A. F. Ziegler, and R. J. Eckroade. 1999. Evaluation of disinfectants with the addition of antifreezing compounds against nonpathogenic H7N2 avian influenza virus. Avian Dis. 43:533–537.

Galvin, S., A. Dolan, O. Cahill, S. Daniels, and H. Humphreys. 2012. Microbial monitoring of the hospital environment: why and how? J. Hosp. Infect. 82:143–151.

Kang, M., J. He, T. Song, S. Rutherford, J. Wu, J. Lin, G. Huang, X. Tan, and H. Zhong. 2015. Environmental sampling for avian influenza A(H7N9) in live-poultry markets in Guangdong, China. PLoS ONE. 10:e0126335.

Musavian, H. S., T. M. Butt, A. Baltzer Larsen, and N. Krebs. 2015. Combined steam-ultrasound treatment of 2 seconds achieves significant high aerobic coun and Enterobacteriaceae reduction on naturally contaminated food boxes, crates, conveyor belts, and meat knives. J Food Protect. 78:430–435.

Northcutt, J. K., and M. E. Berrang. 2006. Influence of a chicken transport cage-washing systme on wastewater characteristics and bacteria recovery from cage flooring. J. Applied. Poult. Res. 15:457–463.

Spackman, E., D. A. Senne, T. J. Myers, L. L. Bulaga, L. P. Garber, M. L. Perdue, K. Lohman, L. T. Daum, and D. L. Suarez. 2002. Development of a real-time reverse Transcriptase PCR assay for type A influenza virus and the avian H5 and H7 Hemagglutinin Subtypes. Clin. Microbiol. 40:3256–3260.

Wanaratana, S., R. Tantilertcharoen, J. Sasipreeyajan, and S. Pakpinyo. 2010. The inactivation of avian influenza virus subtype H5N1 isolated from chickens in Thailand by chemical and physical treatments. Vet. Microbiol. 140:43–48.