Implications, efficiency and evaluation of cleaning and disinfection in commercial broiler farms

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Introduction

Good hygiene practices on farms can reduce the risk of introduction and persistence of animal diseases and diseases that are transmittable from animals to humans (zoonoses). These infectious agents can not only lead to disease outbreaks resulting in sub optimal production and flock mortality, but also to an increase of veterinary costs and condemnation rates at slaughterhouses as well as animal welfare issues. This all leads to high economic losses for the farmer (Jung and Rautenschlein, 2014) and in case of epidemic diseases, preventive measures such as quarantine or even destruction of animals (Gelaude *et al.*, 2014). It is therefore of great importance to prevent disease outbreaks through biosecurity measures rather than cure them (Gelaude *et al.*, 2014; Laanen *et al.*, 2014). Biosecurity includes all measures preventing pathogens from entering a herd (i.e. external biosecurity) and reducing the spread of pathogens within one herd (i.e. internal biosecurity) (Sarrazin *et al.*, 2014). One aspect of internal biosecurity concerns cleaning and disinfection (C&D). This paper deals with implications, efficiency and evaluation of cleaning and disinfection in commercial broiler farms supplemented with some data from other poultry sectors and pig nursery units.

General aspects on cleaning and disinfection

A good cleaning and disinfection programme consists of 6 steps. The first 4 take place during cleaning and the last 2 during disinfection. Moreover after disinfection in many cases a vacancy period is implemented (Table 1). Finally after C&D, the hygiene status of animal houses is preferred evaluated (step 8). (Luyckx, 2016)

Table 1: 8 steps of an ideal cleaning and disinfection (C&D) and evaluation programme between production rounds. (Luyckx, 2016)

Step	Category	Description
1	Cleaning	Dry cleaning
2		Wet cleaning: washing premises with water
3		Wet cleaning: soaking premises with cleaning product
4		Wet cleaning: rinsing premises with water
5		*Drying*
6	Disinfection	Disinfection of premises
7		*Vacancy*
8	Evaluation	Monitoring the hygiene status after C&D

Sampling and analysis to evaluate cleaning and disinfection

In order to evaluate C&D in animal houses, an evaluation tool was designed by our research group. Sampling techniques such as surface sampling with swabs and agar contact plates (ACP) and air sampling were tested during the successive C&D steps, i.e. before cleaning (BC); after cleaning (AC) and after disinfection (AD), in six broiler houses on two farms. During surface sampling, ten to twelve defined locations were sampled in quadruplicate: floor, air outlet, wall, air inlet, drinking cup, feed pan, feed hopper, pipes, drain hole, loose material, roof and floor crack. The effectiveness of cleaning was investigated by bacteriological analyses on swabs, ACP and air samples; adenosine triphosphate (ATP) hygiene monitoring and a visual inspection. The effectiveness of disinfection was examined by bacteriological analyses on swabs, ACP and air samples. In addition, surface and air samples were taken before cleaning to determine the initial bacteriological status of the broiler houses. On swab and air samples and on ACP, enumerations of total aerobic flora, *Enterococcus* spp. (hygiene indicator) and *Escherichia* coli (hygiene indicator and index organism for *Salmonella*) was carried out. In addition, an enrichment of swab and air samples was carried out for the detection of *E. coli* and *Salmonella*.

The results of the study showed that ACP were found to be less suitable than swabs for enumeration. In addition to measuring total aerobic flora, *Enterococcus* spp. seemed to be a better hygiene indicator to evaluate C&D protocols than *E. coli*. All broiler houses were *Salmonella* negative, but the detection of its index organism *E. coli* provided additional information for evaluating C&D protocols. ATP analyses gave additional information about the hygiene level of the different sampling points.

In conclusion, the evaluation tool that provides valuable information for evaluating C&D protocols consists of: ACP for total aerobic flora counts AD; swab enumeration for total aerobic flora and *Enterococcus* spp. BC, AC and AD; and the detection of *E. coli* on those swab samples. After cleaning, ATP analyses could also be carried out for additional information about the hygiene status of the different locations.

In addition to the evaluation tool, the dynamics of the different bacteriological parameters was examined. It was shown that the mean total aerobic flora determined by swab samples decreased from 7.7 + 1.4 to 5.7 + 1.2 log colony forming units (CFU)/625 cm² after cleaning and to 4.2 + 1.6 log CFU/625 cm² after disinfection. Surprisingly, total aerobic flora was significantly reduced by an average of 1.5 log after the disinfection step, which was less than the 2 log reduction obtained by cleaning ($P< 0.01$) which indicates the importance of a good cleaning prior to the disinfection and that in practice, a 5 log reduction, a European Standard (EN1656) that needs to be fulfilled by the validation of disinfectants, is far from achieved during disinfection for total aerobic flora in a practical setting of broiler houses. More details on these results can be found in Luyckx *et al.* (2015a).

On farm-comparisons of different cleaning protocols in broiler houses

The final evaluation tool mentioned in above paragraphs and in Luyckx *et al.* (2015a) was used to evaluate the effectiveness of four cleaning protocols: the difference between whether or not applying an overnight soaking step after dry cleaning and/or the use of warm (60 °C) or cold water during cleaning was studied. Two to three C&D rounds were evaluated in 12 broiler houses on five farms. Total aerobic flora and *Enterococcus* spp. enumerations on swab samples showed that cleaning protocols preceded by an overnight soaking step with water, caused a greater bacterial reduction compared to protocols without a preceding soaking step. No differences were found between protocols using cold or warm water during cleaning. When analysing ACP for total aerobic flora counts, taken AD, no differences were found between protocols. Which demonstrates the lesser sensitivity of the technique.

Additionally, statistical analyses showed that sampling 10-12 locations (see Luyckx *et al.* 2015a) in one fold (compared to 4 fold) per broiler house was sufficient to evaluate C&D. This means that costs and working time can be reduced for future research on evaluating C&D methods. Furthermore, a comparison between power consumption and working time of the four protocols was carried out. When broiler houses were cleaned with warm water, less water and working time were spent in comparison with protocols using cold water. Although broiler houses were soaked with water overnight, water consumption was still lower than when houses were cleaned without a preceding soaking step. This means that a preceding soaking step reduced the amount of water needed to clean broiler houses afterwards. In addition, working time spent on cleaning after soaking was less than cleaning without a preceding soaking step. However, it should be taken into account that soaking of broiler houses can be time consuming by postponing the high pressure cleaning.

Finally, locations that are difficult to clean and possible sources of infection were identified. Drinking cups, drain holes and floor cracks were identified as most critical locations for C&D in broiler houses, while feed hoppers and roofs were identified as the cleanest. More details on these results are outlined in Luyckx *et al.* (2015b).

Optimalisation of cleaning and disinfection poultry houses based on hygienogram scores

Hygienogram scores in Belgium

In order to quantitatively estimate the hygienic status of poultry houses (broilers, laying hens and breeding poultry) in Belgium, hygiene monitoring after C&D between two production rounds is performed by an official body (e.g. DGZ) or by the veterinarian through standard sampling schedules on regular occasions.

From 1998 until 2013 sampling for hygienograms after C&D was legally obliged at poultry houses according to the health qualification and production type. From 2013, there was no longer a legal requirement to take a routine hygienogram at poultry houses with the exception of a *Salmonella* positive flock. Only members of the Belgian quality system 'Belplume' (www.belplume.be) are obliged to take a hygienogram every 3 production rounds, with a yearly minimum of 2 hygienograms per stable for broilers and each production round for laying hens. Hygienogram scores and related data obtained in Flanders during the period 2007 to 2014 from all above mentioned cases were analyzed in our following study.

Hygiene monitoring is performed 24 to 72 hours (h) after disinfection of the poultry house by impressing surfaces with RODAC (Replicate Organism Detection And Counting) plates containing plate count agar (PCA) for 15 seconds. The agar also contained a neutralizing solution (RODAC, PL-agar, P309.16.0017.025) to neutralize the residual action of the disinfectants on the microbiological growth. Different sampling points are sampled 1 to 6 times, depending on the type of housing and capacity of the farm (Table 2). In total, 23 RODAC samples were collected per stable. Samples are transported under cooled conditions to the laboratory on the same day. On arrival in the laboratory, RODAC-plates are incubated upside down at 37 ± 1 °C for 18 to 24 h followed by enumeration of the TAC. Plates overgrown by moulds were considered 'improper' and not suitable for further analysis. To each TAC-value a hygiene score between 0 and 5 is assigned (

Table 1), corresponding with very good and very bad respectively. Based on the 23 hygiene scores for each sampling point the final hygienogram score is calculated as the arithmetic mean. (Maertens *et al.* 2018)

Table 2: Surfaces to be sampled for hygiene monitoring after C&D using RODAC-plates containing PCA + neutralizing solution, for each type of Flemish poultry housing system (Maertens et al. 2018).

Floor house				**Cage system ***				**Barn and aviary system**			
		Small capacity		Production		Rearing		Production		Rearing	
Sampling point	N	Sampling point	N	Sampling point	N	Sampling point	N	Sampling point	N	Sampling point	N
Floor	6	Floor	6	Floor corridor	2	Floor corridor	2	Floor	4	Floor	4
Feed system	4	Feed system	4	Feed system	4	Feed system	4	Feed system	3	Feed system	4
Drinking system	4	Drinking system	4	Drinking system	3	Drinking system	4	Drinking system	3	Drinking system	4
Wall	3	Wall	3	Cage wall / surface	3 / 3	Cage wall / surface	4 / 4	Wall	2	Wall	2
Ceiling	2	Ceiling	3	Ceiling	2	Ceiling	2	Ceiling	2	Ceiling	2
Inside air inlet	2	Inside air inlet	2	Inside air inlet	1	Inside air inlet	1	Inside air inlet	1	Inside air inlet	1
Feed hopper	1	Feed hopper	1	Feed hopper	1	Feed hopper	1	Feed hopper	1	Feed hopper	1
Floor anteroom	1			Floor anteroom	1	Floor anteroom	1	Floor anteroom	1	Floor anteroom	1
				Egg belt	2			Laying nest	3	Slatted platform	4
				Egg collecting area	1			Egg collecting area	1		
								Slatted platform	2		
Total	**23**		**23**		**23**		**23**		**23**		**23**

N, number of samples; *, Battery (banned in the EU from January 2012) / furnished cage

Table 1: Hygiene scores for the individual sampling points corresponding to the number of total aerobic counts (Maertens et al. 2018)

Total aerobic count / plate	Hygiene score
0	0
1 – 40	1
41 – 120	2
121 – 400	3
> 400	4
Not countable	5

Study of the hygienogram scores in Belgium

The study analyzed the hygienogram scores of 19739 poultry flocks in Belgium after C&D. Scores were obtained during the period 2007 to 2014. Data relating to the C&D protocol i.e. year, season, husbandry system, production type, cleaning product, sampler, active components of the disinfectant, disinfection time, disinfection temperature and disinfection responsible were also collected.

The average hygienogram score decreased significantly over time suggesting a general improvement of hygiene obtained after C&D between 2007 and 2014. Differences in scores were found between the husbandry systems; with barn/aviary system having a significant better hygienogram score compared to floor house, furnished cage and battery. Significantly better scores were also found when a cleaning product was used in the C&D protocol (Figure 1). Disinfection with a peracetic acid and hydrogen peroxide combination or formaldehyde gave the best scores (Figure 2). In addition, C&D protocols using ≥ 2 different disinfectants showed improved results compared to the use of 1 single disinfectant. Finally, disinfection applied by a specialist contractor resulted in a better score compared to disinfection done by the farmer. In conclusion, analysis of the hygienogram scores and related data allowed to identify several factors resulting in an improvement, which may reduce the total bacterial load in poultry stables, and consequently the number of zoonotic and pathogenic micro-organisms. More details on results can be found in Maertens *et al.* (2018).

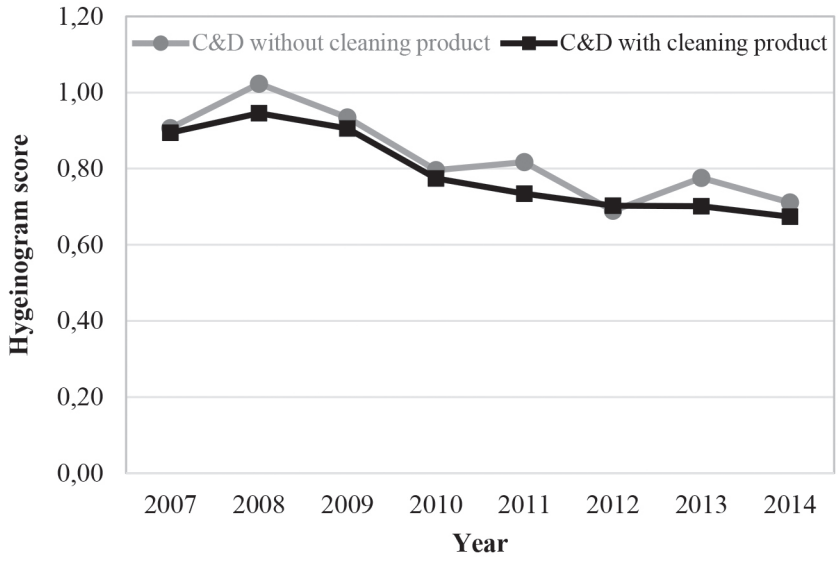

Figure 1: Hygienogram score for C&D protocols carried out with or without a cleaning product for the period 2007 to 2014 (Maertens et al. 2018)

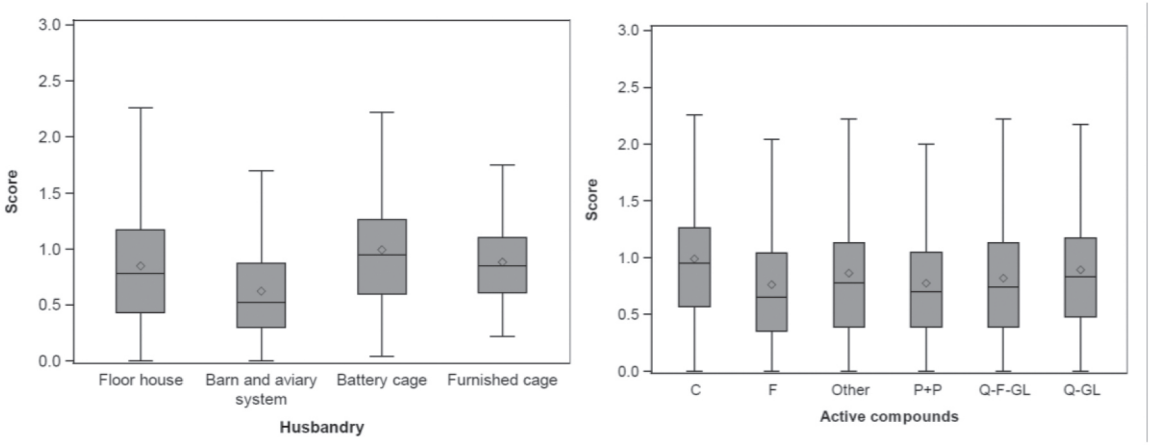

Figure 2a: Hygienogram score after C&D different husbandry systems
Figure 2b: Hygienogram score after disinfection with different active disinfection compounds (C = Chlorine, F = Formaldehyde, P+P = Peracetic acid & hydrogen peroxide, Q = Quats, GL = Glutaraldehyde)

Residual dominant microflora after C&D and disinfectant susceptibility in broiler houses

Also the residual dominant microbiota after C&D was identified in broiler houses. Therefore, sampling was carried out in 4 broiler houses on a pilot farm AC and AD. The used disinfectant was based on hydrogen peroxide and peracetic acid. Enumerations were carried out for total aerobic flora, *Enterococcus* spp. and *Enterobacteriaceae* on Plate Count Agar (PCA), Slanetz and Bartley (S&B) and Violet Red Bile Glucose Agar (VRBGA), respectively. The dominant microbiota was assessed by (GTG)5 analysis and 16S rRNA gene sequence analysis. In addition, minimum bactericidal concentration (MBC) tests were carried out on 18 selected isolates belonging to the *Enterobacteriaceae* family and 10 *Enterococcus faecium* isolates, to determine the susceptibility of these isolates against the used disinfectant. A great variety of bacteria was detected. In total, 363 and 255 isolates were identified AC and AD, respectively. The most dominant bacteria belonged to *Brevibacterium*, *Brachybacterium* and *Staphylococcus* AC and Bacillus, *Brevibacterium* and *Staphylococcus* AD. In addition, on both sampling moments, *Enterococcus faecium* was dominant amongst the *Enterococcus* spp. isolates. On the selective medium for *Enterobacteriaceae*, genera Enterobacter and *Pantoea* and *Aeromonas* (non *Enterobacteriaceae*) were dominant AC and *Escherichia*, *Lelliottia* and *Pantoea* AD. In addition, pathogenic species for poultry and humans were identified not only AC but also AD. MBC results showed no obvious trend in selection of less susceptible isolates for the used disinfectant AD compared to AC. These results indicate the non-existence of disinfection resistance of the remaining flora after disinfection. In addition, the results showed that *Enterobacteriaceae* isolates are less susceptible to the used disinfectant than *Enterococcus faecium* isolates. Further details on this study are outlined in Luyckx *et al.* (2017).

Biofilms in drinking water systems of broiler houses

Water quality in the drinking water system (DWS) plays an important role in the general health and performance of broiler chickens. Conditions in the DWS of broilers are ideal for microbial biofilm formation. Since pathogens might reside within these biofilms, they serve as potential source of waterborne transmission of pathogens to livestock and humans. Knowledge about the presence, importance and composition of biofilms in the DWS of broilers is largely missing. In this study, we therefore aimed to monitor the occurrence, and chemically and microbiologically characterise biofilms in the DWS of five broiler farms.

The bacterial load after the application of disinfectants in DWSs was determined by enumerations of total aerobic flora (TAC) and Pseudomonas spp. The dominant flora was identified and their biofilm-forming capacity was evaluated. Also, proteins, carbohydrates and uronic acids were quantified to analyse the presence of extracellular polymeric substances of biofilms.

Despite the application of disinfectants in the water and the DWS, average TAC was still 6.03±1.53 log CFU/20cm². Enumerations for Pseudomonas spp. were on average 0.88 log CFU/20cm² lower. The most identified dominant species from TAC were *Stenotrophomonas maltophilia*, *Pseudomonas geniculata* and *Pseudomonas aeruginosa*. However at species level, most of the identified microorganisms were farm specific. Almost all the isolates belonging to the three most abundant species were strong biofilm producers. Overall, 92% of all tested microorganisms were able to form biofilm under lab conditions. Furthermore, 63% of the DWS surfaces appeared to be contaminated with microorganisms combined with at least one of the analysed chemical components, which is indicative for the presence of biofilm. It is clear that the intended disinfection in DWS is not achieved and it is better to speak of sanitisation in this situation.

The three earlier mentioned dominant species are considered as opportunistic pathogens and could consequently be a potential risk for animal health. Additionally, the biofilm-forming capacity of these organisms could promote attachment of other pathogens such as *Campylobacter* spp. and *Salmonella* spp. Therefore more research is done by our research group concerning the interaction between these

pathogens and microorganisms originating from DWS. Detailed results of our study on the occurrence and characterization of biofilms in drinking water systems of broiler houses can be found in Maes *et al.* (2019).

Competitive exclusion and vacancy period in pig nursery units

Notwithstanding tested in pig nursery units following results are also usefull for the poultry sector to know. A competitive exclusion (CE) protocol was compared against a classical C&D protocol in pig nursery units. CE protocol consisted of microbial cleaning (Bacillus spp. spores, enzymes and detergent) and spraying the Bacillus spp. spores during down-time (after cleaning) and production. Sampling was performed: immediately after pig removal; 24 h after cleaning (CE units) or disinfection (control units) and after 1 week and 5 weeks of production (piglets present). On these samples, analyses of bacterial spores, *Enterococcus* spp., (haemolytic) *E. coli*, faecal coliforms, methicillin resistant *Staphylococcus aureus* (MRSA) and *Salmonella* were performed. In addition to the bacterial analyses, feed conversion, faecal consistency and antibiotic use were monitored. Analyses of haemolytic *E. coli*, *E. coli* (index organism for *Salmonella*) and MRSA showed that the infection pressure after CE cleaning was not reduced to the same extent after classical C&D during down-time. Therefore, we can assume that no improvement of pathogen elimination is noticed. In contrast, young piglets have a greater chance of being infected when arriving in these CE units. In addition, no improvement in hygiene was found: during the 2nd and 3rd production round, higher *Enterococcus* spp. (hygiene indicator) enumerations were found than after the 1st production round and no differences in faecal coliforms contamination between the two types of units were found. In addition, no difference in feed conversion nor faecal consistency (indicator for gut infections) of piglets raised in CE and control units was seen. Finally, also no differences in treatments with antibiotics was found. So CE experiments with probiotic-type bacteria did not meet the claims provided by the manufacturer. Further details on this study are outlined in Luyckx *et al.* (2016).

Also the effect of a longer vacancy period after cleaning and disinfection in pig nursery units was studied. The results in this study suggest that a vacancy period of 10 days after disinfection without any extra hygienic measures does not give added benefit in the hygiene protocol of nursery units compared to shorter vacancy periods. (Luyckx *et al.*, 2016).

To conclude ….

Our studies showed the importance of a good cleaning step in the reduction of bacteria during C&D as the cleaning step was able to reduce the overall contamination level even more than the disinfection step. Therefore, it is important to continue to evaluate commonly used as well as alternative cleaning protocols in order to lower the infection pressure and optimise hygiene on farms.

Critical locations during C&D are drinking nipples, floor cracks and drain holes in broiler houses.

Besides, we showed that an overnight soaking step before high pressure cleaning contributes to the efficacy of cleaning. In addition, no difference between cleaning with cold and warm water of broiler houses was shown.

Significantly better hygienescores are obtained when a cleaning product was used in the C&D protocol. Disinfection with a peracetic acid and hydrogen peroxide combination or formaldehyde gave the best scores. In addition, C&D protocols using ≥ 2 different disinfectants showed improved results compared to the use of 1 single disinfectant. Disinfection applied by a specialist contractor resulted in a better score compared to disinfection done by the farmer.

It was shown that a great variety in bacteria, including several pathogens, are still present after cleaning and disinfection. This survival may be due to the presence of remaining organic material and diluting water or the use of insufficient concentrations but not due to disinfectant resistance of the concerned bacteria.

Despite cleaning and regular application of oxidising disinfection products, sampled surfaces on the inside of DWS in broiler houses showed rather high microbiological counts and the presence of biofilms in 63% of the sampled surfaces. It is clear that the intended C&D is not achieved and it is better to speak of sanitisation in this situation. Even without *Salmonella* spp. and *Campylobacter* spp. being detected it is known that biofilm could play a role in the maintenance of these pathogens in the DWS of broiler chickens. Therefore more research need to be done in a biofilm model system concerning the interaction between these pathogens and microorganisms originating from DWS.

As alternative protocol, a competitive exclusion (CE) method was shown no to be a valuable alternative in animal houses (tested in pig nursery units), possible due to the highly soiled conditions. Finally, implementing a vacancy period of 10 days or less after C&D in pig nursery units, without any extra biosecurity measures, does not further decrease bacteria.

Several manuals with guidelines for implying a good and complete hygiene management on farms are already available for the poultry. It is beneficial to replenish these information sources with the results obtained in our studies.

Briefly, a good cleaning and disinfection protocol should consist of (i) dry cleaning, (ii) an overnight soaking step with water, (iii) washing with water, (iv) soaking with a detergent, (v) rinsing with water, (vi) a drying step and (vii) disinfection, while extra attention should be paid for the critical locations. After C&D, it is advised to monitor the hygiene status of several locations, including the critical locations.

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