

Effective use of disinfectants in disease prevention and control: II

With the exception of cases involving ovarian infection, the hatching egg is free of micro-organisms when it leaves the oviduct. This presumes a clean cloaca, that is not affected by diarrhoea, since wet droppings will contaminate the shell.

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Then it may face many challenges. If the egg is laid in the nest (floor eggs and eggs laid on slats are 'dirty' by definition), a dirty nest or litter will immediately contaminate the egg, as will dirty egg belts in the case of mechanical or automatic nests. Thus, eggs laid in nests or transported on belts contaminated with droppings, broken eggs, wet shavings etc may be as contaminated as floor eggs are, or even more.

Next, non-sanitised hands may put them on contaminated flats, trays or cartons. The egg storage room in the breeder farm also stores many micro-organisms as does the truck that brings them to the hatchery. Once there, they may again be contaminated. Moreover, a 'rough ride' to the hatchery can cause cracks in the shell, that allow for an easier bacteria penetration and affect hatchability. Needless to say, the vehicle transporting the eggs should be clean and disinfected.

In addition to all that, other biological vectors such as insects, rodents or wild birds can contaminate the eggs should they be in contact with them.

Last but not least, wide temperature changes can make the eggs sweat, which allows rapid multiplication and easier access of micro-organisms to the highly nutritious egg content.

So, there is room for hygiene measures at each of the above 'critical control' points. In addition, frequent egg collection will help reduce contamination (and 'spontaneous incubation' in hot climates).

As a summary, the precious hatching eggs are at risk from:

- External contamination of the shell and through pores and hairline cracks.
- Vertical transmission (from infected flocks).
- Internal contamination (of yolk and albumen).
- External vectors such as hands, trays, vermin, transport equipment, etc.

Nature provides the egg with some natural barriers such as the cuticle, the calcium carbonate shell (with up to 17,000 pores), the two shell membranes, and chemical defences within the albumen.

The cuticle is a physical barrier to micro-organisms, but it can be removed by improper washing products and procedures or by rubbing the egg. The shell thickness and pore length will determine the resistance of shell penetration.

Bacteria will penetrate thin shelled eggs (from older flocks) more easily. Calcium should be added to the feed of breeder hens, but be sure that enough calcium is absorbed in the metabolic process to ensure a strong shell formation.

Hens with diarrhoea will not absorb sufficient nutrients and calcium, and more will be excreted. Chemical sanitisers will reduce the microbial population on the shell at the time of application.

However, if micro-organisms have already invaded the egg, it is too late; the sanitisers will be ineffective.

Egg washing or not?

An egg can appear clean, but can carry over 100,000 micro-organisms on the shell surface. A test conducted by the Institute of Poultry Research in Beekbergen, Holland, indicated that mechanical egg washing can reduce the counts to 50 per egg. 'Nest clean eggs' are generally accepted as having less than 10 CFU bacteria and <5 fungi/13cm².

Washing only dirty eggs is not the total answer, as clean eggs can be re-contaminated in the setters by bacteria from the un-washed eggs which appear clean.

Dry cleaning hatching eggs, e.g.

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| Nest hygiene | Clean nests and belts, regularly disinfected |
| Egg collection | With clean hands and flats, regularly disinfected |
| Egg storage in farm | Cool (around 18°C/65°F) in a clean and disinfected area |
| Egg transport to hatchery | In a cleaned and disinfected truck, with good (air) suspension |
| Egg storage in the hatchery | Cool (around 18°C/65°F) in a cleaned and disinfected egg room |
| Egg handling in general | Carefully, not causing cracks |

Table 1. Hatching egg hygiene before setting (eggs from a healthy flock).

with paper, will remove the cuticle that protects them and is therefore not advisable.

The University of Athens, Georgia (USA) reports that 'the difference in hatchability between nest clean and dirty eggs was due to higher embryonic mortality following transfer into the hatcher of dirty eggs'. So, washing dirty hatching eggs is not a luxury after all.

The biggest problem with egg washing (and disinfection) is that there should be proper temperature and concentration control.

Hatching eggs can be washed with alkaline products (based on potassium hydroxide), to remove mainly fat and protein, which can be either chlorinated or non-chlorinated. Proper temperature control (42-45°C or 108-113°F) is crucial.

The water should be warmer than the egg contents throughout the cleaning cycle. This will result in a positive pressure in the egg, causing the inner membrane to expand against the shell to help prevent anything from entering the egg. Contact time should be limited to approximately five minutes, in order not to damage the cuticle. The washing machine should be temperature and concentration controlled (automatically stopping when either are not optimal).

Disinfection afterwards should be at a slightly higher temperature, to prevent the product from entering the pores (45-47°C or 113-117°F); after rinsing at the same temperature. Use only disinfectants chemically compatible with the cleaning prod-

uct. There must be no conflict between the surfactants in the cleaning product (anionic surfactants neutralise cationic).

Use only recommended disinfecting products at the correct concentration or penetration of the cuticle can occur. Ideally, a disinfectant with a residual action should be used, to prevent early re-contamination. This is not the case with formaldehyde fumigation (that does not kill the spores of *Aspergillus fumigatus* neither).

Fogging of hatching eggs with a QAC/glut based disinfectant has shown, based on large scale trials (done by CID LINES on 9.7 million broiler hatching eggs) that there are alternatives to formaldehyde fumigation, that have at least the same CFU reduction (actually better) and did not affect hatchability. The key is to have the right nozzle, with the correct droplet size.

Table 1 gives a summary of the major actions to be taken.

Setters and setter room biosecurity

Obviously, single stage setters allow for a thorough clean out and disinfection after every 18 days.

Nevertheless, a setter room with single stage setters is still a multi-stage operation that normally never stops running. There are eggs from many flocks, there are embryos in different stages of development. So even in a setter room with single

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stage incubators, cross contamination from setter to setter may occur if air can flow from one setter to another.

The setter room is considered the 'clean zone' (provided the eggs have been disinfected prior to setting). However, exploders (often caused by *Pseudomonas*) can cause serious contamination. They are the biggest threat of the clean zone in the hatchery.

The incubators not only incubate embryos, but also many bacteria. In multi-stage setters, this growth of bacteria is uninterrupted, unless regular spray or mist disinfection is carried out and exploded eggs are removed and their debris is cleared up. Fumigation in the setter with formaldehyde, a carcinogenic product, cannot be done between 24 and 96 hours of embryo development. Moreover, formaldehyde has no residual action so does not prevent recontamination.

Remember that bacteria can double every 20 minutes. Table 2 shows what bacteria need for growth and how we can reduce this growth.

Floors, walls and setters can be washed with a 'universal' cleaner, designed specifically to remove the typical debris of the 'clean zone' (yolk, albumen, blood).

This detergent should also be suitable for application with a foam lance or scrubbing machine and therefore have good adhesion. In the case of hard water, it is advisable to alternate once per month with an acid foam to descale surfaces.

A good foam formulation will cling to ceilings and vertical surfaces, allowing a longer contact time for the chemical to act.

The terminal disinfection should

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| Food | Yolk and albumen | Remove waste, keep clean |
| Water | In egg and via humidifier | Avoid leakage, treat water |
| Temperature | In incubator | Store eggs in cool, and clean area |
| Shelter | In eggshell, fittings, ducts | Clean and disinfect eggs, rooms, machines and equipment |
| Air | Ventilation of machines and building | Spray or fog in machines, mist in rooms, renew air filters |

Table 2. Bacterial needs, sources and solutions.

also be versatile enough to be applied by spraying, foaming and fogging. Room fogging (or misting) in the setter (and hatcher) rooms allows the product to enter the machines through the air inlets and to disinfect the incubators at the same time.

Hatcher and hatcher room biosecurity

In the dirty zone (hatcher room, chick room, wash room, reception and storage of dirty boxes), stronger cleaning products are advised; especially for cleaning the hatchers and plenums, where lots of fluff needs to be removed, (*salmonella* can live for years in fluff).

An alkaline foaming detergent, or even better an alkaline gel with higher viscosity will do the job properly. Instead of relying on 'elbow grease', it is better to rely on the chemistry of specially designed products, allowing for a long enough contact time and thus saving on water consumption, energy costs and cleaning time. Again, it is advisable to rotate on a monthly basis with an acid foaming detergent. Especially in the dirty zone, it

is important to follow the correct procedures:

- Remove all visible debris manually (with shovel and brush).
- High pressure wash with (foaming) detergent (by foam lance).
- Rinse.
- Allow to dry.
- Disinfect.

Often, step four is forgotten. When the disinfectant is sprayed on a wet surface, it may become diluted more than it should.

Moreover, the surface tension of water that is still present in cracks and holes will impede good penetration of the disinfectant solution (even if it does contain surfactants). A well formulated product with good surfactants will penetrate dry cracks more easily.

You will have noticed that there is no need for rinsing the disinfectant from the hatcher cabinet. When the product has a residual action, you can simply spray, or (even better) foam it on all surfaces, load in the transferred eggs and close the doors. The product will keep on working throughout the hatching process as long as it does not evaporate.

The germ counts increase logarithmically when the chicks start

pipping. Excellent field results have been observed by fogging a QAC/glut based disinfectant in hatcher cabinets, compared to formalin.

The use of a 'plenum' or 'fluff tunnel' behind the hatchers (equally to be cleaned and disinfected after every hatch) avoids fluff re-entering other machines or just flying around. Hence the importance of negative pressure in the 'dirty area'.

Chick room and wash room biosecurity

Automation equipment can be washed and disinfected like the hatchers.

Trays, crates and baskets can be washed with alkaline detergents, eventually chlorinated (which will sanitise them and bleach white baskets). It is important that the products do not foam when washing through tunnel machines. If the temperature drops (near the end of the cycle) proteins will cause the formation of foam. So, temperatures should be high enough to remove the organic matter (60°C and more or 140°F and more) but not so high as to damage the plastic

(as of 90°C or 194°F) and not too high to neutralise the chlorine in case of chlorinated tray wash either.

Ideally, these alkaline products which remove mainly fat and proteins should be rotated with an acid, non-foaming detergent to remove mineral deposits (lime-scale, iron), egg shell residues and residues from the alkaline cleaners.

The acid product will unblock the nozzles and de-scale the inside of the tunnel washer. However, it is advisable to disinfect the interior of the tunnel washer by spraying and even to wash your washer regularly.

Hatcher baskets and chick boxes should be disinfected immediately after washing by spraying. If setter trolleys and trays go back to the farm, they must be disinfected. If farm buggies are being used, they should equally be disinfected.

When a vacuum waste removal and silo system is not available, the ofal containers also need cleaning (with a universal detergent) and disinfecting afterwards.

Vehicle biosecurity

Vehicles transporting hatching eggs, day old chicks, feed, broilers, manure and meat are crossing all over the place worldwide.

Vehicles are 'mobile vectors': they can bring contact between the 'source' (the reservoir) and the 'target' (the bird or the egg) and get contaminated 'on the road'.

Trucks should have been washed (inside and outside) and disinfected after every delivery, whether it is hatching eggs, chick, broilers or feed transport. A slightly alkaline, foaming truck shampoo should remove the outside 'traffic film' (a build up of dust, grease, petroleum and exhaust residues, dead insects) and the same product for cleaning the setter can be used for cleaning inside the vehicle. This product should not be corrosive (and definitely not contain chlorine or be too concentrated on sodium hydroxide that corrode aluminium).

Ideally, the truck shampoo should be applied as a foam, starting from the bottom and going up with the lance. Wash vehicles in the sun should be avoided.

After cleaning, the vehicle should be rinsed off. Start at the bottom, going up and move the pressure lance from left to right. Apply a final rinse from the front towards the end, to remove the remaining foam before disinfecting.

The disinfectant should obviously be not corrosive (it should ideally have been tested by an automotive organisation) and it should equally work in cold temperatures where they occur.

Livestock transport has often been defined as the primary disease vector, such as in the recent AI out-

breaks on three continents and in the FMD outbreak in the UK some years ago.

Often, critical places are forgotten, such as the underneath of the vehicle, the inside of the wheel arches and the driver's cabin (livestock trucks are usually not designed to be easily cleaned).

Equally important is the replenishment of farm gate wheel dips. Last but not least, we have observed wheel disinfectant pads that were smaller than the circumference of the vehicle's wheels.

Automatic spraying installations, reaching the underneath and the arches deliver a better job. They also assure 'fresh' disinfectant to be used. But they cannot operate when freezing.

When freezing, manual disinfection is required. Eventually, a glycol mixture (a good anti-freeze agent) can be added to the water to dilute the disinfectant, provided the disinfectant is compatible with glycols.

If plastic chick boxes are used, they usually go back to the hatchery. There, they can be washed (and disinfected) in the tray washing machine (tunnel). The same procedure is required as for hatcher trays.

The other vector are obviously the crates in which the broilers are being transported to the processing plant.

Research on 72 containers which had been used for transporting 12 flocks has shown that broilers leaving the farm salmonella free, can be positive when arriving at the processing plant.

Farm biosecurity

Apart from rodent control and insect control (to be done immediately after bird removal), we will strictly focus on cleaning and disinfection.

Procedures

The Dutch ICC (Integrated Chain Control) system describes the procedures for poultry houses as follows:

- Remove litter, empty drinkers and clean dry all visible dirt.
- Wash down with a cleaning agent and allow for enough contact time (20 minutes) and clean drinker lines (and flush them afterwards).
- Rinse and let dry.
- Disinfect (by spray or foam; foaming will visualise better where the product has been applied and stays longer on vertical surfaces and ceilings, allowing for a longer residual action).
- Install new litter, re-install and fill the feeders and drinkers.
- Do a terminal disinfection by (thermo) fogging. Do a continuous disinfection of trucks (wheel dips),

people (hand hygiene, foot dips) and drinking water.

In some countries, like the USA, the reality is far away from this concept and litter is used time and time again, sometimes called 'dirt floors'. But then again, the University of Georgia writes: 'Because most US broiler houses have dirt floors, *Clostridium perfringens* has the opportunity to flourish and cause physiological problems to the birds, high flock mortality rates and financial losses for the farmer and the poultry company'.

A clean out and disinfection of the feed silos is equally advised. With foot dips, regular replacement of the disinfectant is important and washing the boots first!

Product characteristics

The cleaning agent should be alkaline and ideally applied as a foam, allowing for better contact time. It should also be chemically compatible with the disinfectant (anionic surfactants neutralise cationic ones).

Drinking water treatment

The FAO recognises that water contaminated with faeces from infected birds is an important source of infection of AI. Surface water (from rivers, ponds) is unacceptable unless it is properly treated. Open header tanks (where wild birds and rodents have access to) is as bad. Even if your water quality is acceptable at source, it can still be contaminated in the lines because of the biofilm. The further towards the end, the more risk of microbial contamination.

Cleaning of water lines means removing the scale and the biofilm. The biofilm is a polysaccharide layer, caused by adding vitamins, medication etc. through the water. It harbours mainly enterobacteria (salmonella, *E. coli*) but also virus like AI. It impedes the good functioning of medicine, vaccines, etc. It will, as scale, block the nipples and reduce the water flow. Chlorine (that gets neutralised by organic matter) will not remove the scale and not even penetrate the biofilm.

Moreover, the efficacy of chlorine depends on the pH. NaOCl dissociates in water into hypochlorous acid (HOCl) and hypochlorite ion ($-OCl$). Only the first one is really effective (80-300 times more) and fast (100 times faster) at killing bacteria (the negative charge of OCl⁻ creates an obstacle to penetrating the cell of a micro-organism).

Maximum efficacy is obtained at pH 5 or lower, because the lower the pH, the more HOCl is available. Moreover, the lower the temperature, the less HOCl there will be.

'Total chlorine' is the sum of free

chlorine (HOCl plus OCl⁻) with chloramines. Free chlorine is not considered effective unless it is 85% hypochlorous acid, which means the pH should be under seven at all times.

Removing the biofilm is only possible by oxidation. Stabilised hydrogen peroxide does the job. And, if the product does not contain heavy metals (like silver), it can also be given during production until the last day, avoiding a new build up and sanitising the drinking water, provided it has the proper registrations (PT 5 in Europe).

Its efficacy is hardly affected by the pH, and when it is stabilised, it will reach the end of the line.

Stabilised H₂O₂ works in seconds. All this without leaving residues in the meat or eggs: it dissociates in H₂O and O₂, two natural elements. Even in 'favourable circumstances' (low pH, no organic matter, no minerals) chlorine will still need 10-15 minutes contact time at 0.1-0.2mg/L to kill bacteria and needs a higher concentration (0.3-0.5mg/L) and 30-45 minutes to kill viruses.

Chlorine may leave residuals such as THM (Tri Halo Methanes) described as 'possible cancer causing agents'. Moreover, organic matter makes it lose its microbiocidal properties, so it can no longer act as a disinfectant.

Conclusions

In this article, and in part one, which was published in International Hatchery Practice Volume 31 Number 2, we have followed the embryo from the breeder farm through the hatchery, to the farm.

Every phase needs an integrated biosecurity approach with different products and different procedures. But there, it is not over.

The stringent rules of processing biosecurity, storage biosecurity, transport biosecurity and biosecurity at the processing plant and during transport and storage in the shop will have to be implemented as well. Always pay attention to the four V's: visitors, veterinarians, vehicles and vermin.

Last but not least, basic hygiene rules will have to be implemented to take the piece of poultry meat home or to the restaurant, to store it and to prepare it.

Chicken is safe, as long as it is cooked.

The AI virus can live:

- Endlessly in frozen chicken meat.
- 1 month in cooled meat.
- <2 hours at >56°C.
- <10 minutes at >60°C.
- <1 second at >88°C.

When sufficiently cooked, other micro-organisms, such as salmonella, will be eliminated as well. When prepared in a biosecure way, chicken is a safe meal. ■