Aspergillus prevention in hatcheries with Clinafarm® Elanco
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Aspergillus: a worldwide hatchery problem

Aspergillosis is caused by contamination with fungi of the genus *Aspergillus* and results in considerable economic loss in the poultry industry worldwide. *Aspergillus* is a fast-growing mould that only requires warmth, a little moisture and plenty of organic material to thrive. The fungi are found everywhere in the environment, from tightly closed jam jars, to the sterile environment of an operating theatre.

Over the past few decades, modern hatcheries have undergone dramatic changes. Chick output has risen nearly tenfold, and production of one to three million a week or more is no longer exceptional.

The increasing concentration of breeding poultry, hatching eggs and broiler chicks has contributed to continuous exposure to significant numbers of fungal spores.

But it would be hard to find a more ideal breeding ground than the modern poultry production chain and hatcheries in particular.
Chapter 1: The problem

Respiratory Integrity is the normal functioning of the respiratory system that allows the bird to reach their full genetic potential.

The fungal pathogen
The most common *Aspergillus* moulds found in poultry and game bird farming are *Aspergillus fumigatus* and *Aspergillus flavus*. *A. fumigatus* grows easily on organic material such as eggs, down and poultry litter, while *A. flavus* is more likely to grow on feed grains such as wheat, maize, oats and rye. Other *Aspergillus* moulds, such as *A. niger*, *A. nidulans* and *A. versicolor*, are also seen in poultry production. *Aspergillus* spores have a diameter of 2.5 microns, far too small to be seen by the naked eye. A small speck of dirt or faecal material on an egg can contain many thousands of spores which may each grow into a mature mould, producing spores and spreading contamination. For example, a single gram of chick fluff may contain up to 190,000 mould spores.

**Decreased hatchability**
One of the consequences of aspergillosis in a hatchery is decreased egg hatchability, due to environmental infection of the egg. Embryos die at about 16 days of incubation, as a result of spores entering the egg via a porous shell or a thin hairline crack.

This does not necessarily mean that the eggs were contaminated at the hatchery: the spores may have arrived with the eggs. Nevertheless, the hatchery is seen as the main proliferation site. There are constant opportunities for entry and production of spores and all hatcheries undergo a continual or seasonal challenge from *Aspergillus*. 
1.1 The clinical signs

Newly hatched chicks: respiratory and nervous signs

In hatched chicks, infection occurs after the inhalation of large numbers of spores from a contaminated hatchery environment. Chicks are particularly vulnerable during the first three days of life, as their respiratory systems are too immature to fight off the infection. Chicks often become infected immediately after hatching. This leads to mortality in the first ten days after hatching. When inhaled by a newborn chick, the spores will cause lesions in the respiratory tract, leading to breathing difficulties. Necropsy of infected chicks reveals yellow clumps of fungi in the trachea, air sacs and lungs.

Chicks may also have difficulty walking if the fungus has gained access to the central nervous system. High early mortality may occur and surviving chicks do not grow well. Birds infected subclinically may show no obvious signs but will have reduced weight gain, increased feed conversion and/or higher condemnation rates at the slaughterhouse.

Acute brooder pneumonia: high mortality in hatcheries

The acute form of aspergillosis, also called brooder pneumonia, is mainly seen in hatcheries and on broiler farms. Over 90% is hatchery related. It is characterised by severe outbreaks among young chicks, with high mortality rates (up to 30%) in the first 10 days of life. Respiratory signs include dyspnoea, yawning, rapid breathing and stretched necks. Secondary infections due to bacteria such as E. coli or salmonella, causing systemic disease, are not uncommon. Surviving chicks will often perform poorly later in life.

The chronic form: more common in adult birds

The more chronic form of aspergillosis is usually seen in broilers from 4-5 weeks of age and in breeder farms, especially in turkeys. The disease most commonly develops on contaminated litter, particularly if it is deep, damp and matted with faeces. Feed or other organic material may also be a source of contamination. The chronic form is often manifest as secondary infections that develop in birds that are already weak or diseased.

In addition to the classical respiratory symptoms seen in chicks, there may also be spread to other organs, and Aspergillus can cause systemic infection, dermatitis, osteomyelitis, ophthalmitis, encephalitis and polyserositis, including arthritis. Typical post-mortem findings are granulomatous nodules in the lungs and air sacs. These ‘aspergillomas’ contain inflammatory cells and fungal hyphae. Plaques of caseous and mucopurulent exudate may be seen in the trachea and on air sac membranes at necropsy.
1.2 Diagnosis

Aspergillus spores are present everywhere in the environment, and are carried on air currents until they come into contact with an organic growth substrate. The prevention of aspergillosis in hatcheries requires appropriate monitoring of the presence of spores in the environment. Samples may be taken at various locations and from various sources.

Air sampling

Routine sampling of the air in hatcheries is a simple and inexpensive method to test for the presence of Aspergillus spp. Agar plates with specific growth media such as Sabouraud dextrose agar are readily available. These media may include an antibiotic such as chloramphenicol to reduce the contamination of the plates by airborne bacteria. Cycloheximide should be avoided as this may inhibit Aspergillus growth.

Plates are placed open in the area to be sampled, with the lids next to them, facing downwards (to avoid the collection of dust and spores). After ten minutes, the lids are replaced and sealed to the plates with adhesive tape. The sampling location is marked on the bottom of the plate, which is then incubated, upside down, to reduce condensation on the agar. After 36-48 hours, the mould colonies can be counted.

Aspergillus fumigatus, the most common of the Aspergillus moulds found in hatcheries, have a blue-green appearance with a white border. The base of the mould growth, on the underside of the plate, is colourless or yellowish white. Immature Aspergillus moulds are small and white. If these are found, the plate should be incubated for another 5-6 hours after which growth will be more easily recognisable.

Simultaneous laboratory tests may help you recognise Aspergillus fumigatus by sight with reasonable accuracy. However, even if moulds of another, non-pathogenic variety are found, this indicates that environmental conditions in hatcheries are ideal for mould proliferation - of any kind.
**Egg necropsy**

The presence of *Aspergillus* moulds can also be detected in eggs or birds. Egg necropsy is often carried out routinely to check flock fertility, embryo mortality, malposition or malformation. In order to determine the degree of fungal contamination, hatcheries should perform egg necropsy on unhatched eggs every hatch day. At day 21, unhatched eggs from a few baskets per hatcher should be opened to examine the air cells for mould growth. Ideally, eggs from each supplier breeding flock are included. Before breaking, the eggs should be closely examined for cracks or porosity of the shell.

Eggs found to contain small typical greenish-blue moulds due to *A. fumigatus* at necropsy were probably contaminated three to five days earlier, most likely during transfer or *in-ovo* vaccination. This mould growth is easily visible in unfertilised or early dead embryonated eggs. If the egg was contaminated at an early stage, e.g. at breeder farm level, the eggs will be very light following evaporation of the egg contents due to a porous or hairline-cracked shell. The air chamber will be large and may be filled with blackened spores. If embryonic death occurred, contamination with *Aspergillus* and environmental bacteria will have lead to breakdown of the egg content, leading to a putrid debris in the egg. All eggs containing mould should be recorded to monitor the contamination source and trends. Necropsy should be carried out outside the hatchery to prevent contamination if infected eggs are found. A protective mask should be worn.

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**Do’s and don’ts of air sampling**

**Do:**
- Sample regularly to follow contamination trends.
- Sample in the same places under the same conditions to allow comparison.
- Sample hatchers only when clean, empty, dry and at working temperature, in order to check the efficacy of hygiene procedures.
- Have a laboratory test carried out initially to identify Aspergillus moulds.
- Inform all hatchery operators of the time and place of sampling to avoid breakage or disturbance of plates.
- Check results from agar plates away from the hatching area to avoid contamination.
- Wear a mask when examining the results, as Aspergillus spores are potentially harmful.
- Record the results and take pictures for easy comparison.

**Do not:**
- Place sample plates in areas of activity, near doorways or ventilators as the air turbulence may influence the colony count.
- Sample during washing or disinfection procedures, as disinfectants and detergents may have an impact on the agar and subsequent counting or identification.
**Eggshell sampling**

The direct sampling of eggs allows an early warning of a build-up of *Aspergillus* at the breeding farm. Although this type of sample does not give accurate colony counts, it may be indicative of a problem at breeder farm level, in particular if egg samples from the same flock are consistently positive for *Aspergillus* growth.

Eggshell sampling is easy: a cross is drawn with a felt tip pen on the base of an agar plate, dividing the plate in four quarters. Remove the lid of the plate and lightly touch the tops of 4 eggs on the growth medium, one on each segment. Note the name of the flock on the base of the plate and incubate as described previously.

**Surface sampling**

A sterile swab is used to take a sample of dust from a surface such as a ventilation grill or hatcher exhaust, preferably when clean. Not only will a dirty area almost invariably prove positive, but sampling of clean areas allows you to check the efficacy of cleaning procedures. Although this test will not give accurate colony counts, it is indicative of hygiene measures.

After sampling, an X or W is drawn with the swab across the agar surface of a plate. Mark the position of sample area and incubate. Mould growing on certain surfaces such as wood or cardboard may not always be easy to recognise, and should be swabbed and cultured for identification.

**Under the microscope**

Lesions of dead birds can be sampled and sample material can be cultured and examined under a microscope, as well as samples from eggs or environmental cultures. Under the microscope, the septate hyphae and typical, dandelion-like conidia of *Aspergillus* may be visible. The hyphae can be stained using lactophenol cotton blue. Alternatively, first a potassium hydroxide solution may be used before staining. When using necropsy material, *Aspergillus* conidia with spores will only be found in body compartments open to the outside air, such as the respiratory system.

The various *Aspergillus* species can be identified on the basis of their microscopic and cultural properties.
1.3 *Aspergillus* and aflatoxin

*Aspergillus* can grow on stale, damp poultry feed and produce the mycotoxin known as aflatoxin. When feed contaminated with aflatoxin is consumed by the breeders, this may lead to immunosuppression, a drop in egg production and reduced fertility/hatchability of the eggs.

Aflatoxin production may occur at the feed mill or even earlier, in grain stores on the farm of origin. Feed may also become contaminated in bulk feed bins on the breeding farm if these are not emptied prior to each new feed delivery. The design of certain bulk bins may cause feed remnants to lodge in nooks or ledges, thereby contaminating the fresh feed delivery.

Conditions in feed bins are ideal for *Aspergillus* growth and sporulation, as they are subject to temperature changes (hot during the day, cool at night) and moisture due to condensation. Bagged feed and additives should be stored in a clean, dry area.

1.4 Impact on the production chain

The main origin of *Aspergillus* contamination at hatchery level is the breeder farm.

Control at the breeding farm begins with good litter control, nest box hygiene, more frequent egg collection, careful egg grading and removing all damaged and dirty eggs. Eggs should be stored under hygienic conditions and transported to the hatchery in clean vehicles, driven with care. Poor hygiene or insufficient antifungal treatment will perpetuate an *Aspergillus* contamination in the hatchery. Hatcheries in turn are a potential source of aspergillosis problems at the broiler farm. Poor performance or even clinical signs and mortality may be seen in young broilers that were infected as chicks.

Hygienic measures and antifungal treatment at all levels are therefore essential.
Chapter 2: *Aspergillus* and aspergillosis: in the field

2.1 Hatcheries: the perfect breeding ground

It would be hard to find a more ideal breeding ground for *Aspergillus* than the modern poultry production chain, in particular at hatchery level, where moisture, temperature and nutrients create an ideal environment for the growth of *Aspergillus*.

Spores are easily transported by light air currents, such as wind or ventilation. Additionally, the concentration of hatching eggs and chicks leads to permanent exposure to significant numbers of fungal spores and recontamination.

**The incubator: ideal conditions for *Aspergillus***

*Aspergillus* requires no specific conditions for growth or germination. The spores thrive in a wide range of temperatures (12-65°C), moisture and pH. Optimal growth is achieved at temperatures of 37-45°C and high humidity. In other words, an incubator is an ideal growth environment.

Growth and sporulation require a cycle of warm ‘wet and dry’ conditions: wet conditions for growth and dry for sporulation. Together with a growth medium such as egg yolk or other organic material (wood, cardboard, chick fluff), an ideal breeding environment for *Aspergillus* is created.
**Ventilation systems: difficult to clean**
The ‘wet-dry’ cycle is also maintained by the chick take-off (dry) followed by washing and cleaning of the hatcher (wet). Combined with active ventilation, this may explain the often high *Aspergillus* proliferation in hatchery ventilation systems. As they are often difficult to clean routinely, ventilation, filters, shafts and exhausts are a continuous source of recontamination.

**Evaporative coolers**
These devices, often roof mounted, use a pad which has cool water pumped across and the hot air from outside the hatchery is pulled through the pad. This reduces the temperature of the air by trapping the heat of the air in the pad. Any organic debris can be trapped inside the pad. At night or during periods of cooler weather, these coolers are often not used. Consequently, this allows the pads and any organic matter to dry out, thus perpetuating the wet-dry cycle needed for growth and sporulation of *Aspergillus*.

**External sources**
Although less common, outside sources of *Aspergillus* contamination are also possible. These include pollinating trees in the area, dust from harvesting corn or other field crops, dust from feed mills or wood processing plants. Even cutting the grass around a hatchery is not without risk! Spores can be drawn into the hatchery via the ventilation systems and contaminate the internal ventilation units.
2.2 The modern hatchery: a portrait

The development of modern hatcheries in the past few decades has been significant. Chick output from hatcheries has risen from a typical average of 100,000 - 400,000 to over 2 to 3 million per week in some hatcheries.

Production rhythm

The production rhythm has changed, too. While thirty years ago, most hatcheries would hatch two days per week and transfer the eggs for two days, leaving a day for cleaning and disinfection, modern hatcheries will hatch 4 to 6 days per week. This also means that all major operations, such as the setting of eggs, the transfer from setter to hatcher and the take-off or ‘pulling’ of chicks, can all take place on the same day, increasing the risk of cross-contamination.

From multiple to single stage

While previously hatcheries were multi-stage — with incubators containing eggs of different ages and flock origin — modern hatcheries are single stage: an incubator will only contain eggs of the same age and sometimes origin. This allows conditions to be optimised to match the needs of the specific age of the embryo, rather than providing average conditions for embryos of all ages. This also means that the incubators can be thoroughly cleaned and disinfected every 18 days (between batches of eggs).
Modern building design has followed food factory standards and incubator cabinets, walls, floors and most equipment now have easy-to-clean plastic or stainless steel surfaces. Ducts of electrical cables, water and compressed air are built into walls or ceilings to reduce the areas gathering dust.

Many hatcheries have plenum chambers to exhaust the air laden with dirty fluff from hacters, allowing easy and efficient cleaning. Most hatcheries have installed positive pressure ventilation to prevent dust from moving into clean areas. However, this system obviously only works if all the doors in a hatchery are kept closed.

Improved biosecurity: essential
Modern hatcheries apply strict biosecurity measures, and all employees and visitors are required to shower and change before entering. The one-way circulation of people, air, products and drains is becoming standard practice, with different employees working in ‘dirty’ and ‘clean’ areas of the building. All fresh air coming into the hatchery is filtered to prevent the introduction of *Aspergillus* spores. However, a filter is only effective if regular cleaning or replacement is carried out. Contaminated filters may turn into a reservoir for mould spores.

Many premises are also equipped with vehicle washing/disinfecting equipment at the entry to the hatchery compound. Modern cleaning chemicals are used, usually degreasing foam detergents, followed by power washing and specific treatment. Egg washing
machines, when used correctly, further help to reduce the bacterial and fungal contamination of dirty eggs.

**Increased automation: a potential source of contamination**

Increasing manpower costs have triggered a wave of automation in hatcheries. Operations such as egg transfer, candling, *in-ovo* vaccination, chick separation and counting have largely become automated. This also means that all eggs or chicks pass through the same equipment, which may become a source of infection if not regularly and effectively cleaned and disinfected.

Increased vigilance is required as badly adjusted transfer machines may break eggs or cause hairline cracks during handling, while the vacuum heads, pipes and filters may become contaminated, spreading mould and bacteria from egg to egg.

While candling machines effectively identify and remove infertile eggs at transfer, the waste container may become a potential source of contamination if not strictly separated from the transfer area.

Chick separators may stir up large volumes of dust, while chick counters may cause stress and/or damage to the chicks.

**In-ovo vaccination: optimal hygiene required**

Automated *in-ovo* vaccination has dramatically increased the speed and efficiency of vaccination in hatcheries. However, it may also be a potential source of *Aspergillus* infection, in a contaminated environment, as the opening created by vaccination may allow spores to enter and grow on the air cell membranes. *In-ovo* vaccination should therefore be carried out under strict hygienic conditions: clean, uncontaminated air, aseptic vaccine mixing procedures and strict cleaning and antifungal disinfection procedures.

Most *in-ovo* vaccination machines have specialised apparatus and procedures for cleaning their own integrated transfers and the manufacturer’s recommendations should be strictly followed.

Main *Aspergillus* risk factors at hatchery level:

- Contaminated, cracked or dirty eggs coming in from the breeding farm.
- Contaminated ventilators and filters.
- Badly adjusted transfer machines (damaged eggs, source of contamination).
- Unhygienic *in-ovo* vaccination procedures.
- Contaminated vacuum heads and pipes.
- Waste container for infected eggs.
- Badly adjusted chick separators/counters (dust and stress).
- Reuse of insufficiently cleaned equipment.
- Contaminated cardboard or wooden material.
- Poor biosecurity (insufficient hygiene, open doors etc.).
- Poor hatchery design: no separation between ‘dirty’ and ‘clean’ areas.
- The use of disinfectants with limited antifungal activity.
2.3 The economic impact
The main consequences of aspergillosis contamination are major economic losses at hatchery level due to lower hatching rates and chick mortality. Chicks that were contaminated early in life will turn into broilers with a decreased performance, a higher mortality rate and a possible source of environmental contamination. The disease can also damage the hatcheries reputation and give rise to complaints from broiler and breeder farms.

The overall cost of a major *Aspergillus* infection is due to:

- Increased embryo mortality (especially at day 16), causing decreased hatch.
- Early chick mortality (up to 50% in worst cases).
- Reduced viability of chicks, immunosuppression.
- Poor broiler weight gain.
- Increased mortality during the growth period.
- Increased cost to overcome infection.
- Loss of customer confidence in chicks.

**The benefit from Clinafarm® disinfection**
Although fortunately a full-blown aspergillosis outbreak is not very common, most hatcheries will suffer from the subclinical effects of aspergillosis in broiler flocks. This may go unnoticed in the absence of high mortality rates, but poor growth in a flock, lack of vitality and uneven growth are all signs of a subclinical aspergillosis infection.

*Aspergillus* contamination has a significant impact on the technical and financial results of a hatchery, and a Clinafarm® disinfection programme is a justifiable investment. The return on investment of a Clinafarm® program can easily be achieved by a 0.5% improvement in hatchability.

**No cure, only prevention**
Treatment of the disease is virtually impossible and has no economic value to commercial producers.

Once a hatchery is contaminated it is impossible to get rid of *Aspergillus* completely due to the ease of growth of the pathogen and the favourable growing conditions at the hatchery. While antifungal treatment will allow you to control the problem, it is highly unlikely that it can be removed completely and permanently. However, strategically designed sanitation programmes should be able to effectively control the environmental infection pressure and prevent substantial economic losses.

Therefore, this should be a strategic focus of hatchery managers.
2.4 Case histories

Case 1

Poor hatchery management = poor broiler performance A hatchery had noticed a higher than average early mortality of chicks placed. Managers on the growing farms were also complaining that the finished birds were not achieving their target growth weights and showed a lack of uniformity.

Sampling at the hatchery showed that egg storage, incubation rooms, hatcher rooms and chick handling rooms were all heavily contaminated by *Aspergillus*. Investigation also revealed a lack of hygiene (insufficient cleaning and disinfection) and biosecurity (doors left open between ‘dirty’ and ‘clean’ areas). The hatchery manager explained that this was necessary as there had been problems with air availability for the hatchers.

The chick holding room was also used for box storage where new chick and egg boxes were stored on wooden pallets prior to assembly. Investigation revealed that the boxes at the back had been stored for several years, with new deliveries stacked in front. There were also large quantities of damp chick fluff under the pallets. This was heavily contaminated with *Aspergillus* as were the old damp boxes.

Every time chicks were stored in this room, fans were used to circulate air and cool the chicks. Coupled with the open doors, this enabled *Aspergillus* to colonise the whole hatchery.

Although it was impossible to quantify the losses, the growing farms had been complaining for a long period about mortality and decreased performance. However, it is certain that an extra box store and an appropriate ventilation system would have largely covered the losses.

Case 2

A small mistake with major potential consequences

A well-managed, modern hatchery plant with an efficient ventilation system and single-stage incubation, had noticed a rise in *Aspergillus* counts during routine sampling. This coincided with a sharp rise in temperature during a particularly hot summer. Examination of unhatched eggs showed a small increase in air cell mould.

On hot days, the hatchery would remove some of the filters in the ventilation system to allow better airflow and cooling. This had been done for many years and had never caused a problem. Furthermore, recorded sampling results showed that no increase in mould counts were noted previously following the dates when filters were removed. The entire ventilation system was cleaned weekly.
Further investigation revealed a waste skip outside the hatchery walls, containing macerated hatchery waste from the previous days’ hatch. The waste skip was 3 metres away from the hatchery air inlet grill.

Normally, the waste skip was situated on the other side of the hatchery close to the macerator and the exhaust air outlets. Due to increased waste removal charges, the waste was only collected every other day instead of daily. To avoid causing a nuisance due to the smell of decomposition, it was decided to move the skip, once full, to the shaded side of the side of the hatchery - where the air inlet happened to be located. This was done in particular on hot days - the same days the filters were removed. Sampling of the waste skip showed very high contamination due to the waste company not washing and disinfecting the empty skips before delivery to the hatchery. Due to the good management practice of regular sampling and record keeping, the mistake was quickly rectified before a major problem could occur.

Case 3
How minor equipment malfunction jeopardised healthy eggs
A hatchery which used egg necropsy noticed an increase in the incidence of mould on egg air cells. There was also an increase in eggs with bacterial contamination (rotten eggs). It seemed to be worse in particular trays. However, it did not appear to point to a problem with particular setters, hatchers or even particular flocks and seemed to be completely random. There were no obvious suggestions as to why the increase in *Aspergillus* and bacterial counts could be occurring. The on farm egg stores were checked and samples taken, these gave positive results but as *Aspergillus* is regularly found in the dust on poultry farms it was not thought to be the cause of the problem since *Aspergillus* has to penetrate the shell of the eggs to cause air cell growth.

It was noted that many of the eggs were grossly contaminated with *Aspergillus* which pointed to contamination on farm or early in incubation. Again egg storage was examined. The farm egg stores were fairly primitive, no refrigeration or humidifiers were used, the egg stores were insulated and eggs were collected from the farms on a daily basis during the summer period when the problem was noted. The daily collection during summer was to get the eggs cooled as rapidly as possible using the refrigerated egg stores in the hatchery.

During sampling of the hatchery egg store it was noticed that there was a large wet patch on the floor in front of a humidifier. This had not been
noticed during previous checks as the area had been filled with trolleys of eggs at those times. Examination of the humidifier showed that the atomisation was not working properly due to a build up of calcium carbonate and the humidifier was actually spraying a fine spray of water over the warm eggs which had been brought from the farm. The position of eggs in the store was entirely random which meant that eggs from a different flock would be in this area every few days.

These eggs fresh from the farm and very warm were being rapidly cooled to 16 °C on entering the store and rapid cooling creates contraction, which has a suction effect. This, combined with the wetting of the egg surface, was causing both *Aspergillus* spores and other micro-organisms to be drawn through pores in the egg shells (particularly affecting eggs with porous shells) contaminating the eggs. Thirty minutes of maintenance work on the humidifier cured a problem that took weeks to identify.

**Case 4**

**How a cheap repair led to major losses**

A hatchery using *in-ovo* vaccination found mould in a large number of vaccinated eggs. The *in-ovo* vaccination equipment and integrated transfer were sampled on a regular basis and were not suspected. The hatchers were then sampled and showed either nil or very light contamination. Attention was then directed to the transfer room where *in-ovo* vaccination and the transfer of eggs was carried out simultaneously with transfer only of non injected eggs using an old vacuum transfer machine.

Sampling of the ventilation system was negative, but as soon as work started in the transfer room, sampling showed very high spore counts. Furthermore, it was revealed that the transfer head of the old machine had developed a vacuum leak the previous year, causing eggs to drop. The leak had been fixed by an operator by sealing the vacuum head permanently to its mounting plate using a silicone sealant. When cleaning the machine, the staff would just wash the vacuum cups at an angle using a pressure washer, leaving the transfer head in situ.

After removing the transfer head from the mounting plate it was found to be full of *Aspergillus* mould growing on egg yolk which had been sucked into the transfer by the vacuum. Every time this machine was used, it filled the transfer room environment with spores.
3.1 Clinafarm®, a unique fungicidal disinfectant

Even when strict sanitary precautions are in place, hatcheries will still be confronted with Aspergillus. A strong, specific disinfectant that is effective against this fungus is therefore required. From the wide range of safe antimycotics developed by Janssen Animal Health, enilconazole was chosen as the most appropriate fungicidal disinfectant.

Enilconazole has a very high level of activity against the Aspergillus fungi and its spores, and is therefore highly suitable as a fungicidal disinfectant. A spray formulation and a smoke generator are available, which may be used separately or in combination, depending on the circumstances.

Two presentations: spray and smoke

Clinafarm® spray is particularly suited for the spraying or fogging of large surfaces such as walls, floors and tools. Clinafarm® spray is an emulsifiable concentrate containing 15% enilconazole (150 g/l).

When mixed with water, Clinafarm® spray forms a stable microemulsion. It has high bioavailability for the fungi and spores and potent activity due to the low particle size of enilconazole. The product’s excipients have been selected to improve contact with surfaces, mould and spores, while the oil-based carrier provides a long-term effect - even in areas with high organic content.

Clinafarm® smoke is a ready-to-use enilconazole smoke generator for fumigation of well closed rooms, laboratories or small spaces such as incubators and silos. Clinafarm® smoke contains 5 grams of enilconazole. Clinafarm® smoke generators may be stored for up to two years in a cool place. The smoke is non-toxic to humans or animals.

3.2 Fungicidal and anti-sporulant

Clinafarm® is a disinfectant with both fungicidal and anti-sporulant properties, especially suited to combat aspergillosis. Many products claim a broad-spectrum efficacy against microorganisms and moulds. However, they all have weaknesses when used in the hatchery environment.

For example:

**Formaldehyde**: not effective against mould or spores in normal applications under common hatchery conditions, highly toxic to both humans and animals, in particular to 3 to 8-day old embryos. Carcinogenic.
**Quaternary ammonium:** inconsistent activity against *Aspergillus*, ineffective against spores.

**Lodine-based disinfectants:** not very effective, possible adverse effects on hatchability in high concentrations.

**Chlorine-based disinfectants:** not effective against mould, unstable during storage and use. Highly corrosive. No residual effect. Should not be fogged.

**Phenolics:** variable activity against moulds and spores. Incompatible with certain detergents and other treatments. Toxic. Cannot be fogged.

**Glutaraldehyde:** ineffective against *Aspergillus* and spores, moderately toxic. Cannot be fogged.

**Hydrogen peroxide:** highly corrosive to metals, cannot be fogged.

**Peracetic acid-based disinfectants:** inconsistent effect, corrosive, should not be fogged.

**Ozone:** highly corrosive, should not be fogged.

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### 3.3 Efficient, safe and easy to use

Mode of action

Enilconazole species selectively prevents the formation of ergosterol, which is an essential part of the fungal cell walls.

At extremely low concentrations, Clinafarm® is capable of inhibiting the growth of the most common dermatophytes in animals, and of fungi of the *Aspergillus* type (see table). Enilconazole has broad-spectrum activity and is both fungicidal and anti-sporulant.

When normal safety precautions are followed during application of Clinafarm®, enilconazole has a wide safety margin: no skin or airway irritation in healthy individuals, low absorption in body tissues and no systemic effects.

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**Active in the vapour phase**

<table>
<thead>
<tr>
<th>Fungus species</th>
<th>Inhibitory concentrations in μg/ml</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Complete</td>
</tr>
<tr>
<td>Microsporum canis</td>
<td>1</td>
</tr>
<tr>
<td>Trichophyton mentagrophytes</td>
<td>0.1</td>
</tr>
<tr>
<td>Trichophyton rubrum</td>
<td>1</td>
</tr>
<tr>
<td>Phialophora verrucosa</td>
<td>100</td>
</tr>
<tr>
<td>Cryptococcus neoformans</td>
<td>100</td>
</tr>
<tr>
<td>Candida tropicalis</td>
<td>100</td>
</tr>
<tr>
<td>Candida albicans</td>
<td>100</td>
</tr>
<tr>
<td><em>Aspergillus</em> fumigatus</td>
<td>1</td>
</tr>
<tr>
<td>Sporothrix schenckii</td>
<td>10</td>
</tr>
<tr>
<td>Saprolegnia sp.</td>
<td>10</td>
</tr>
<tr>
<td>Ascosphaera apis</td>
<td>0.1</td>
</tr>
</tbody>
</table>

- : no inhibition of growth
To test the vapour phase activity of enilconazole, a small paper disk impregnated with enilconazole was placed inside the cover of an inverted agar plate 1, allowing no direct contact between the enilconazole disk and the culture medium. Any inhibitory activity observed is entirely due to the gas phase.

As a comparison, another enilconazole paper disk was placed directly on the culture medium of an inverted agar plate 2. Here, the observed activity is due to diffusion.

Both plates were inoculated with Aspergillus. After incubation, both plates showed a central area in the culture medium with total growth inhibition of the fungi and a secondary area where the mycelium had developed, but no spores were formed.

Even in the absence of direct contact, the gas phase activity of enilconazole has a strong activity inhibiting mycelial growth and spore formation.

### Comparative activity of enilconazole and thiabendazole against fungi (minimum inhibitory concentrations in µg/ml)

<table>
<thead>
<tr>
<th></th>
<th>Enilconazole</th>
<th>Thiabendazole</th>
</tr>
</thead>
<tbody>
<tr>
<td>Microsporum canis</td>
<td>1(*)</td>
<td>1(—)</td>
</tr>
<tr>
<td>Trichophyton mentagrophytes</td>
<td>0.1-1</td>
<td>(0.01-0.1)</td>
</tr>
<tr>
<td>Trichophyton rubrum</td>
<td>0.1</td>
<td>(0.01)</td>
</tr>
<tr>
<td>Aspergillus fumigatus</td>
<td>1(—)</td>
<td>(0.1)</td>
</tr>
<tr>
<td>Candida tropicalis</td>
<td>100</td>
<td>—</td>
</tr>
<tr>
<td>Candida albicans</td>
<td>100</td>
<td>(—)</td>
</tr>
</tbody>
</table>

(*) at 1 µg/ml: complete inhibition  (**) ( ): partial inhibition

### A thousand times more active than thiabendazole

Enilconazole is more than a thousand times more active against *Aspergillus* than thiabendazole. It is also more effective against dermatophytes. The growth of most fungi was completely inhibited at concentrations of 1 to 10 mg/l. It is even active against yeasts and, to some extent, against gram-positive bacteria.

**Enilconazole, vapour phase activity**

![Image of agar plates showing vapour phase activity](image-url)
3.4 Clinafarm® spray, a user’s guide
For large areas, surfaces and equipment The Clinafarm® spray formulation contains 150g enilconazole per litre and has an excellent wetting effect on the water repellent spores, giving better contact between the product and the spores. Clinafarm® spray is particularly suited for fogging large areas or for spraying onto walls, floors and equipment. In poultry farms, it can also be used in nestboxes and on litter. The oil-based formulation sticks to surfaces and has a long duration of effect. Clinafarm® spray is less suited for small and confined spaces such as containers or incubators, or in areas that should not get wet, such as laboratories. Clinafarm® spray is also less suited to very high rooms or constructions such as feed silos. In these cases, Clinafarm® smoke should be used.

Effective, non-corrosive and highly compatible with other products
Effective, non-corrosive and highly compatible with other products Clinafarm® spray is highly active against Aspergillus, even in the presence of organic material such as litter, wood shavings, fresh straw and chick fluff. Activity is not influenced by the hardness of the water used to prepare the dilution. It is effective at temperatures ranging from 4-45°C. At temperatures of 25°C or higher, such as in incubators, an increased activity against Aspergillus spp is noted. It is non-corrosive and compatible with many other disinfectants, ectoparasiticides, insecticides and detergents.

Safe for users and animals
Clinafarm® spray is safe for humans or animals, however normal safety precautions should always be followed when applying the product. Clinafarm® spray has a shelf life of five years at room temperature.

WHERE TO USE CLINAFA RM®

Clinafarm® Spray
- For (large) room disinfection: nebulize or fog Clinafarm® Spray
- For surface disinfection: spray Clinafarm® Spray

Clinafarm® Smoke
- Enclosed rooms up to 50m³: Use Clinafarm® Smoke

HOW TO USE CLINAFA RM® SPRAY

Preparation of the solution
- 1 litre of Clinafarm® Spray in 99 litres of water.
- This finished solution (100L) is sufficient for a room of 3000m³ or a surface of 750m².
- For optimal effect, the water should be warm (45°C).
- Spraying or fogging (nebulization) may be carried out with any type of spraying device.
- When diluting the fluid, the type of spraying equipment should be taken into account. The finer the mist or droplets obtained, the smaller the quantity of fluid required.
- in areas with high concentrations of organic material (litter, nest boxes, etc.), the dose may be doubled.
3.5 Clinafarm® smoke, a user’s guide

For confined spaces and less-accessible areas

Each Clinafarm® smoke generator contains 5g of enilconazole, which is released as smoke by a special combustion process. A single generator is sufficient for an area of 50m³. The ready-to-use smoke generator is highly suitable for the disinfection of tightly closed rooms, such as laboratories and incubators, but also feed silos or transport vehicles. Furthermore, it can be used in less accessible locations such as ventilation and air-conditioning systems. No special spraying device is required. Clinafarm® smoke is less suitable for open areas or the disinfection of walls and floors. In these cases, Clinafarm® spray is more appropriate.

QUICK AND EASY

1 Clinafarm smoke for max 50M3 enclosed space.

BEFORE USE

- Empty rooms should be closed for maximum effect: close doors and windows, seal any gaps and switch off the ventilation
- In hatching cabinets in the presence of chicks, the ventilation system should be left on, to allow sufficient air supply and temperature control.

**Instructions for Clinafarm® smoke**

- Remove the lid
- Place the smoke generator on a non inflammable surface (e.g. brick, concrete tile) and away from combustible materials as the container itself may become very hot
- Light the wick with a lighter or match. Within 20 to 40 seconds, the active substance is expelled.
- The generator produces a greyish white smoke without a flame.
- After combustion, the recommendation is to wait at least five minutes for the aluminium container to cool off before touching it with bare hands.
- The smoke should be kept confined in the room as long as possible: at least 30 minutes but ideally 12 hours (overnight).
- Thoroughly ventilate the area after disinfection with Clinafarm® smoke.

Safe for users and animals

Day-old chicks exposed to Clinafarm® smoke in a closed room for 30 minutes, showed no side-effects. (Braem, 1986)

Although the smoke is not toxic, the recommen-
dation is not to inhale the smoke, because of the possible irritating effect of the smoke itself.

Smoke generators have a shelf life of two years at room temperature (< 30°C).

3.6 Prevention rather than cure
Reducing infection pressure at all levels
When controlling aspergillosis in broiler chicks, good hygiene and husbandry at all levels of the production chain are essential. Infection pressure should be reduced to a minimum by proper risk management at all production levels, including breeder and broiler farms.

At breeder farm level, risk factors include:
• poor shell quality due to poor feed quality, disease or ageing flocks
• excessive shell breakage and egg sweating due to poor temperature management
• dirty eggs due to poor hygiene
• inadequate storage or transport

At hatchery level, the risk factors include:
• contaminated, cracked or dirty eggs coming in from the breeding farm
• contaminated or insufficiently cleaned ventilators, filters, equipment, etc.
• badly adjusted transfer machines (damaged eggs, source of contamination)
• unhygienic in-ovo vaccination procedures
• badly adjusted chick separators/counters (dust and stress)
• contaminated cardboard or wooden material
• poor biosecurity (insufficient hygiene, open doors...)
• poor hatchery design: no separation between ‘dirty’ and ‘clean’ areas, the use of disinfectants with limited anti-fungal activity

At broiler farm level, the main risk factors are:
• poor hygiene, increasing the contamination of litter material
• contaminated feed due to feed remnants left behind in the silos (cross-contamination)

Prevention programs
Clinafarm® is of particular interest when used preventively. Broiler producers will note that chicks are of a better quality and are livelier, and that flocks are more uniform.

An aspergillosis prevention programme should involve the use of Clinafarm® spray and smoke in the standard hatchery cleaning and disinfection procedures, including the regular disinfection of the ventilation channels in the hatchery with Clinafarm® smoke generators. The use of Clinafarm® smoke in the egg delivery truck or hatchery fumigation room can further help reduce the incidence of *Aspergillus* on entry of eggs into the hatchery.
4.1 Breeding farms
Breeding farms are the main source of contamination for hatcheries. Eggs carry spore-bearing dust particles on the shell. In the case of porous or cracked eggs, fungal growth may already have started prior to delivery to the hatchery. Contaminated dust will also be present on the setter flats and trolleys if the eggs are graded and placed on setter flats on the farm.

### Table: Clinafarm® treatment in breeder farm

<table>
<thead>
<tr>
<th>Where to disinfect?</th>
<th>When</th>
<th>Clinafarm®</th>
</tr>
</thead>
<tbody>
<tr>
<td>Litter</td>
<td>2 x per month</td>
<td>spray: spray/mist solution 1/50</td>
</tr>
<tr>
<td>Walls and roof</td>
<td>2 x per month</td>
<td>spray: spray/mist solution 1/50</td>
</tr>
<tr>
<td>Nest boxes</td>
<td>2 x per month</td>
<td>spray: spray/mist solution 1/50</td>
</tr>
<tr>
<td>Disinfection of eggs on farm</td>
<td>after collection</td>
<td>smoke: 1 generator/50 m³ (dry)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>spray: spray/mist solution 1/50(wet)</td>
</tr>
<tr>
<td>Feed silos</td>
<td>after emptying and cleaning</td>
<td>smoke: 1 generator/50 m³</td>
</tr>
<tr>
<td>Egg transport</td>
<td>spray or nebulise eggs before</td>
<td>spray: spray/mist solution 1/100</td>
</tr>
<tr>
<td></td>
<td>departure or after cleaning and</td>
<td>smoke: 1 generator/50 m³</td>
</tr>
<tr>
<td></td>
<td>disinfection of truck after</td>
<td></td>
</tr>
<tr>
<td></td>
<td>loading the eggs</td>
<td></td>
</tr>
</tbody>
</table>

4.2 Hatcheries
All areas of the hatchery should be thoroughly cleaned but particular areas will certainly require extra attention. As the spores need organic
Aspergillus contamination pattern

**Aspergillus sp. presence (spores/m$^3$)**

- **DISTRIBUTION**
- **BREEDING FARM**
- **TRANSPORT**
- **RECEPTION**
- **Egg storage**
- **Incubation**
- **Transport**
- **Hatcher**
- **Chick collection**
- **Day 16**

---

**Clinafarm® treatment**

### Clinafarm®

<table>
<thead>
<tr>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>spray: spray/mist solution 1/100 or smoke: 1 generator/50m³</td>
</tr>
<tr>
<td>• considered a ‘dirty’ area</td>
</tr>
<tr>
<td>• avoid pressure hoses</td>
</tr>
<tr>
<td>• remove any cardboard trays</td>
</tr>
<tr>
<td>• avoid spraying directly on eggs</td>
</tr>
<tr>
<td>spray: spray/mist solution 1/100</td>
</tr>
<tr>
<td>• keep multi-stage setters as clean as possible</td>
</tr>
<tr>
<td>• remove cracked eggs</td>
</tr>
<tr>
<td>smoke: 1 generator/50m³</td>
</tr>
<tr>
<td>spray: spray/mist solution 1/100</td>
</tr>
<tr>
<td>• load setters with care</td>
</tr>
<tr>
<td>• have bucket of Clinafarm® liquid available to deposit cracked or broken eggs</td>
</tr>
<tr>
<td>spray: spray/mist solution 1/100</td>
</tr>
<tr>
<td>• remove and clean vacuum cups individually</td>
</tr>
<tr>
<td>• replace or wash air filters</td>
</tr>
<tr>
<td>• observe strict hygiene in case of in-ovo vaccination</td>
</tr>
<tr>
<td>spray: spray/mist solution 1/100</td>
</tr>
<tr>
<td>• major area of contamination</td>
</tr>
<tr>
<td>• clean thoroughly prior to spraying</td>
</tr>
<tr>
<td>smoke: 1 generator/50m³</td>
</tr>
<tr>
<td>spray: spray/mist solution 1/100</td>
</tr>
<tr>
<td>• mix diluted Clinafarm® spray liquid in humidifier bowls</td>
</tr>
<tr>
<td>spray: spray/mist solution 1/100</td>
</tr>
<tr>
<td>smoke: 1 generator/50m³</td>
</tr>
<tr>
<td>spray: solution 1/50</td>
</tr>
<tr>
<td>• add Clinafarm® to circulating water</td>
</tr>
</tbody>
</table>

---

**4.3 Broiler farms**

At broiler farms, aspergillosis outbreaks often occur undiagnosed. Treatment in presence of the animals is possible. In case of problems, the litter, walls and roof should be sprayed with Clinafarm®.

Material as a growth substrate, the egg storage, hatching and chick handling rooms as well as the waste areas are the main sources of contamination. Regular sampling should give an indication of the location of the main *Aspergillus* proliferation sites.
Conclusions

Aspergillosis is a worldwide problem at all stages of the poultry and game bird production chain. Hatcheries are particularly at risk due to the favourable growing conditions for the mould. The main source of infection is contaminated eggs from the breeder farm.

Contaminated hatcheries will observe a drop in hatchability and early mortality, while surviving chicks will show a poor performance later in life at the broiler farm. Infected animals cannot be treated, while contaminated hatcheries can never be decontaminated completely. Good Clinafarm® prevention programmes at all levels of the production chain are therefore essential.

Clinafarm® disinfectant is highly effective against both fungi and spores and offers the poultry industry a unique and versatile tool to prevent and overcome aspergillosis.
For hatcheries: hatching and chick-rooms: nebulize the Clinafarm® spray solution in all rooms at a rate of 10 litres per 3000m3, the night before collecting the chicks; smoke per hatcher the night before collecting the chicks; 1 generator of Clinafarm® smoke per 50m3 may be used. Repetition of the treatment depends on the risk of re-infection.

Directions for use

The dilution generally used for Clinafarm® spray is 1 to 100, i.e.: 100ml of Clinafarm® spray in 9.9 litres of water or 1 litre of Clinafarm® spray in 99 litres of water. Preferably, the water should have a temperature of 45°C. The dosage is 20 mg enilconazole per m2 ground surface; this is 10 litres of the ready-to-use solution per 750m2 or per 3000m3. To use the Clinafarm® smoke generator, first remove the cover and then light the wick.

For rabbit breeding farms:

In farms infected with dermatophytes, the Clinafarm® ready-for-use spray is applied by atomization. The dosage is 50mg per m2 of ground surface, or 25 I of the ready-to-use solution per 750m2 or per 3000m3. To use the Clinafarm® smoke generator, first remove the cover and then light the wick.

For disinfection of other rooms, stables, poultry houses containing litter or nest materials where fungal infection may be found:

Nebulize the Clinafarm® spray solution. The dosage is 50mg enilconazole per m2 or 25 I of the ready-to-use solution per 750m2 or per 3000m3, moistening walls and floors. In rooms which can be closed off well or in sites (e.g. biological laboratories) in which nebulization is excluded for practical reasons, 1 generator of Clinafarm® smoke per 50m3 may be used. Repetition of the treatment depends on the risk of re-infection.

For the disinfection of empty and cleaned rooms and stables where fungal infection may be found:

Nebulize the Clinafarm® spray solution. The dosage is 20 mg enilconazole per m2 or 10 litres of the ready-to-use solution per 750m2 or per 3000 m3, moistening walls and floors. Repetition of the treatment depends on the risk of re-infection.

General remarks

- Clinafarm® smoke is flammable.
- Clinafarm® is non-corrosive.
- Clinafarm® may be mixed with other disinfectants such as formaldehyde.
- As is the case for any disinfection, a thorough mechanical cleaning is required previously.
- After nebulization or spreading of the smoke, it is recommended that the rooms or hatching cabinets be closed as long as possible.
- In case of recurrent infections with skin fungi, due to environmental infection, it may be necessary, besides treating the animals with Imaverol®, to disinfect the environment with Clinafarm®.

Precautions

Do not bring the product in contact with any food or beverages. Avoid inhalation and contact with the skin. Do not eat or smoke during disinfection. After having finished, thoroughly wash your hands. Store the smoke generators in a dry place at room temperature and keep them away from an open flame.

For rabbit breeding farms:

In farms infected with dermatophytes the Clinafarm® ready-for-use spray is applied by atomization. The dosage is 50mg per m2 of ground surface, or 25 I of the ready-to-use solution per 750m2 or per 3000m3. This treatment should be carried out twice a week over the batteries filled with rabbits, and should be continued for at least three weeks. As with any disinfection, a thorough weekly cleaning is absolutely necessary.


Jones M.P., Orroz S.E.; The diagnosis of aspergillosis in birds; Seminars in Avian and exotopic medicine; Vol: 9, 2; 2000; pp. 52-58


S. Department of Bacteriology and Mycology, Janssen Research Foundation, Beerse, Belgium.


Literature reference list on Clinafarm®
# Pips | Flock iD | Examined | CDV_+ | MG_+ | GIX_+ | ARS_+ | LIV_+ | Recommendation
--- | --- | --- | --- | --- | --- | --- | --- | ---

Pipped Embryo Evaluation

Customer Location: ___________________ Hatchery Name: ___________________ Posting Date: ________________________________________
### Embryo Diagnostic Evaluation

<table>
<thead>
<tr>
<th>Farm Name: ______________________</th>
<th>Flock ID: _____________________</th>
<th>Setter No: ___________________</th>
<th>Egg Age: ____________</th>
<th>Hatcher No: ___________________</th>
<th># Eggs Candled: ____________</th>
<th>Eggs/Tray: __________________</th>
<th>% Production: ____________</th>
<th>% Hatch of Flock: __________________</th>
<th>% Hatch of Sample: ____________</th>
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<td>Middle Dead</td>
<td>Late Dead</td>
<td>Live Pip</td>
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<td>Farm Crack</td>
<td>Anomalies</td>
<td>Transfer Crack</td>
<td>Contaminated</td>
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