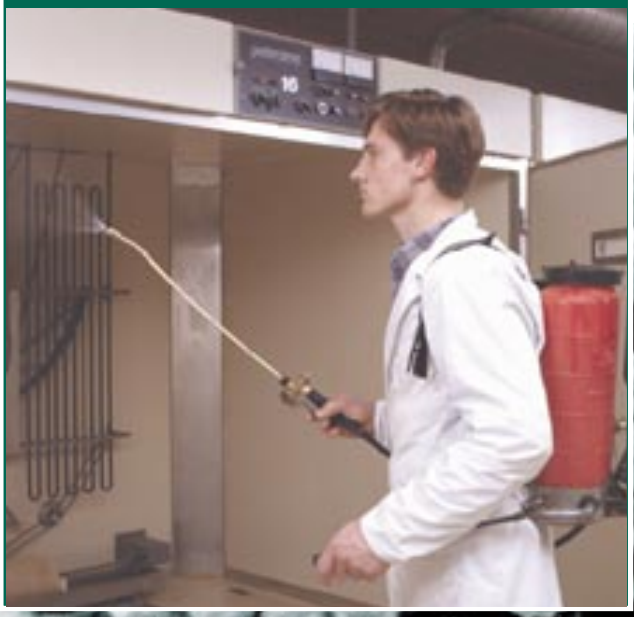
Clinafarm™ Spray



Clinafarm™ Smoke

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There is no cure for aspergillosis...
But there are good ways to prevent it

TRADEMARK

Clinafarm

There is no cure for aspergillosis… But there are good ways to prevent it

Few living entities are so ubiquitous as moulds. The requirements of some moulds are so modest that they can be found everywhere. They can live equally well in a virtually hermetically sealed jam jar or in the so-called sterile environment of an operating theatre.

Moulds and their spores are found not only in the air, soil or water, but also on plants, animals and humans. Whilst the majority of moulds are not harmful to man or animals, constant vigilance must be exercised against those which do present a threat.

Plenty of warmth, a little bit of moisture and a lot of organic material: these are the ideal conditions for *Aspergillus* to flourish. The ideal substrates for *Aspergillus* multiplication are grain, oil seeds and peanuts or mouldy organic material such as hay, straw and compost. The spores of the mould are so light that they are easily wafted about with the lightest breeze. High concentrations can cause disease in susceptible species (mainly birds) and sometimes also in humans.

ASPERGILLOSIS IN HATCHERIES

It would be hard to find a better breeding ground for this mould than the intensive poultry production chain. Concentration of breeder hens, hatching eggs and chick progeny in the typical integrated poultry operations creates an environment for potential exposure to significant numbers of viable fungal spores on a continual basis. The physical environment in the breeder house and the hatchery presents a situation where moisture, temperature and nutrients are ideal for survival and growth of *Aspergillus* spp.

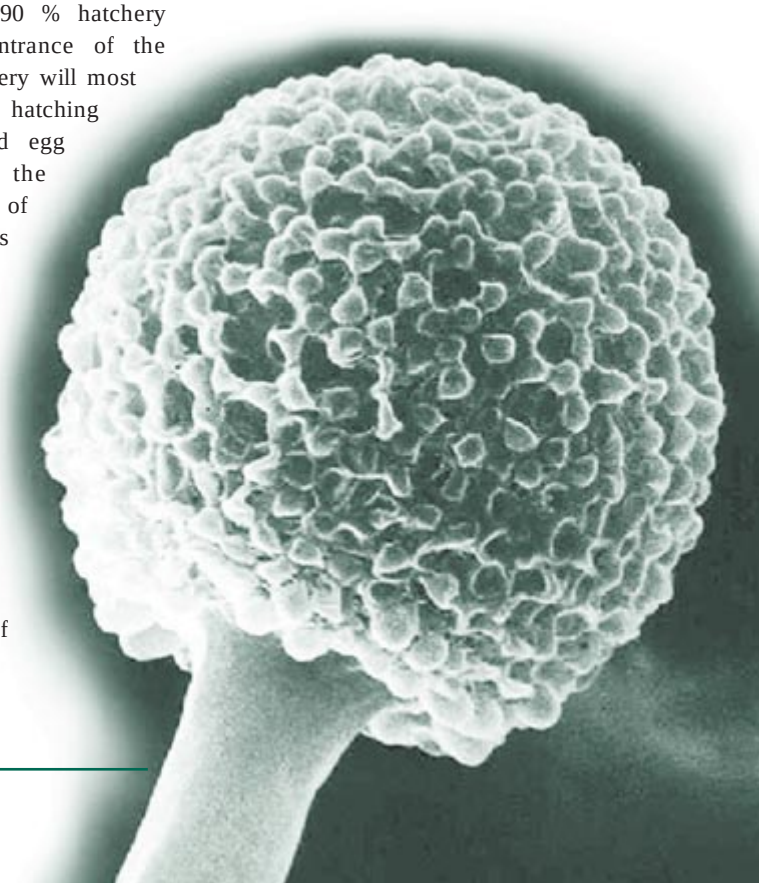
Fungal spores are prevalent in both the breeder and broiler phases. However, the most significant exposure occurs during the incubation and hatching process. It is in hatcheries that aspergillosis can run riot. Millions of *Aspergillus* spores are present in the air, the incubators and the down of the chicks. In general from 14,000 up to 190,000 mould spores per gram of down can be measured in a hatching cabinet. Infection of the hatching eggs does not come about in the oviduct but through contamination after laying. Whether or not an egg becomes infected depends largely on the physical state of the shell. Only dirty and cracked or damaged eggs are attacked. In an infected egg the embryo dies. Once they are infected, cracked eggs spread a dense aerosol of *Aspergillus* spores, which are then inhaled by the chicks during the first few hours after hatching, so that their lungs and air sacs become infected.

Aspergillosis or brooder pneumonia is over 90 % hatchery related. However, entrance of the fungus into the hatchery will most often occur on the hatching egg, setter, flats and egg cases. Obviously, the opportunity for entry of spores is continual as hatching eggs are collected to the hatchery for all breeder flocks at least twice a week.

On a practical basis, nest quality, egg handling, grading, transport and storage also play significant roles in the transmission of *Aspergillus*.

The entry, incidence, transmission and ultimately exposure of the incubating embryos and hatching chicks to *Aspergillus* must be minimised through both the breeder and the hatchery management programmes. In order to decrease the pressure on the hatchery, all procedures – including egg collection, grading, transport and storage, – must be managed for minimum egg sweating, shell breakage and egg filthiness.

Because of the high infection risk in hatcheries, specific strict sanitary measures are necessary to prevent serious problems. The organisation and structure of the hatchery are of central importance: a general principle of one-way traffic must be observed, ensuring that the various handling and transfer routes cross each other as little as possible and egg recontamination is avoided. Any organic material such as dirt on the eggs and trays or chick down will provide the growth substrates needed for this organism to grow and quickly re-contaminate the environment.



This is why it is so important to observe detailed cleaning procedures for all hatchery rooms machines and equipment so they are completely free of any dirt or organic materials. Of special consideration are the ventilation channels and filters. Because the ventilation ducts are more difficult to clean, dust is accumulated and they are all too often a source of continuous recontamination of the hatchery with *Aspergillus* spores.

In hatched chicks, infection usually follows inhalation of large numbers of spores from contaminated hatchery equipment, feed, litter or environment. Chicks are especially vulnerable during the first three days of life. It is very common that chicks become infected immediately after hatching. At the period of hatching the hatching cabinet can be heavily contaminated with spores, which are than inhaled by the newborn chicks.

The chicks temperature is too low to fight off and the cilia in its respiratory system are not yet developed. Clinically infected birds display breathing difficulties and may have to inhale with their necks extended to get enough oxygen. Necroscopy reveals yellow clumps of fungi in the trachea, air sacs or lungs. Signs of central nervous disturbance may be observed if the fungus gains access to the bloodstream and is pumped to the brain. Birds with this problem usually have difficulties walking. Subclinically infected birds can go unnoticed, but have reduced weight gain, increased feed conversion, adverse vaccine reactions, increased condemnation or a combination of these problems.

Aspergillosis or brooder pneumonia may cost an integrator thousands of dollars annually in field performance, veterinary and labour expense, egg destruction and chick mortality.

Treatment of the disease is virtually impossible and serves no economic value to commercial producers. The full attention should be on prevention of the disease with a strategically designed sanitation programme.

PREVENTION RATHER THAN TREATMENT

Even when strict sanitary precautions are taken, hatcheries are still confronted with aspergillosis.

Most disinfectants are not particularly effective against this parasite. Quaternary ammonium chlorides (QAC's) show an inconsistent activity against molds and especially against *Aspergillus* spp.

Glutaraldehyde and formaldehyde are not effective at all against *Aspergillus* spp.

Phenolics have shown to be active against *Aspergillus*, but the efficacy levels depend strongly on the formulation and phenolic that is chosen. A strong specific disinfectant against *Aspergillus* is therefore required to fill the gap that is left with the normal disinfection programme.

From the range of antimycotics developed by the Belgian based company Janssen Pharmaceutica, enilconazole was chosen because it exerts maximal activity against *Aspergillus* spp. Moreover, enilconazole has a vapour-phase effect, permitting disinfection by a particularly simple method based on a smoke generator.

Enilconazole has a very high activity against the *Aspergillus* fungi and against their spores as well. The molecule is therefore well suited for the use as a fungicidal disinfectant. A spray formulation and a smoke generator are available.

The Clinafarm spray formulation was designed to have a very good wetting effect on the hydrophobic (water repellent) spores and so gives better contact. With a sprayer, or fogger the water diluted product is applied to the walls and doors of setters and hatchers and in the rooms of the hatchery.

Clinafarm spray is formulated on an oil-based carrier and has a long persistent activity because it sticks to the sprayed surfaces.

The Clinafarm smoke generators ensure that enilconazole penetrates everywhere, carried by the produced smoke. The generator smokes up in 20 seconds without any flame. Smoke generators are used in closed rooms such as the hatchers. Clinafarm smoke generators are ignited the evening before collection of the chicks. There is no risk for the chicks to coming into contact with the smoke for a short while.

High safety margin: enilconazole is no toxic potential and has been tested in many different animal species. There is no risk if eggs or animals come into contact with the product.

Clinafarm is not corrosive and can be used safely in hatchers and on hatchery of farm equipment.

Mixes with other cleaners: Clinafarm is compatible with most commonly used disinfectants. It may be used along with quaternary ammonium compounds, gluteraldehyde, phenolic compounds and hydrogen peroxide to constitute a convenient on-step sanitation programme.

Enilconazole is 1000 times more potent than thiabendazole: The MIC value (minimum inhibitory concentration) of *Enilconazole* against *Aspergillus fumigatus* is 1 mg/ml whereas this is 1000 mg/ml for thiabendazole.

In a comparative trial between Clinafarm smoke and Fungitec Smoke (thiabendazole) [mycoses 31 (3) 143-147, 1988] it was demonstrated that Clinafarm Smoke is much more potent and excerpts higher levels of efficacy. The results of this trial are summarised in the table below:

Clinafarm disinfection programmes have been tested on a number of commercial chicken, quail and turkey hatcheries. One of the hatcheries had been forced to cease all commercial activities because of continuous re-occurring aspergillosis outbreaks. Despite frequent disinfection with formaldehyde and potassium permanganate, infection with *A. fumigatus* remained a permanent problem. On that farm Clinafarm was nebulized several times with a high-pressure nebulizer. Control samples showed that after some time the remaining infection pressure was very low.

At a quail hatchery, 30% of the chicks died during the first 4 days of life. After treatment, the mortality after

the tenth week was 4%, which may be regarded as normal.

In another example, a turkey hatchery with a capacity of 100,000 poults per week was troubled by poor growth and abnormal spoilage. After treatment of the hatchers and hatching rooms with smoke candles the entire hatchery was negative for *Aspergillus* and the spoilage fell from 2.5% to 1%.

A clear favourable effect has often been observed at less seriously infected farms too. The broiler producers speak of better quality and more lively chicks, more uniform flocks.

Activity of smoke generators containing enilconazole and thiabendazole on spores of Aspergillus fumigatus

Treatment Antifungal	Active dose in g per m^3	Inoculum (standing / hanging)	No. of samples	Mean No, of CFU per ml x 10^3
A. Controls	0	standing	8	95,300 (Extr.:22,400 – 163,000)
enilconazole	0.254	standing	8	6 negatives; 0.3;0.4
enilconazole	0.254	hanging	8	6 negatives; 0.1;0.4
thiabendazole	0.475	hanging	8	2.594 (Extra.: 100 – 8,000)
thiabendazole	0.475	standing	8	12,706 (Extra.: 1,1150 – 23,500)
B. Controls	0	standing	8	95,156 (Extr.: 77,500 – 120,000)
enilconazole	0.146	hanging	8	7 negatives; 3.1
enilconazole	0.146	standing	8	4 negatives; 4 positives 38 (Extr.:22 – 71)
thiabendazole	0.409	hanging	8	10,640 (Extr.: 4,500 – 20,750)
thiabendazole	0.409	standing	8	43,047 (Extr.: 30,000 – 55,000)

PREVENTION PROGRAMME

When designing a prevention programme against aspergillosis in poultry, the sanitary conditions for the whole production chain, from breeder to broiler, have to be considered. The circumstances that can result in subclinical or even clinical aspergillosis problems are manifold.

Some examples:

AT THE BREEDER FARM:

- Poor shell quality due to feeding abnormalities, infectious causes, aging flocks etc.
- Excessive shell breakage and egg sweating due to marginal management or weather extremes.
- Dirty eggs or eggs with extreme high *Aspergillus* contamination due to the poor breeder management conditions.
- Inadequate and too lengthy transport and storage of the eggs

AT THE HATCHERY:

- Insufficient cleaning of hatchery rooms and equipment
- Use of disinfectants with insufficient antifungal activity
- Dirty, cracked and possibly contaminated eggs are not rejected before setting
- Ventilation channels and filters are not sufficiently disinfected and cleaned.
- The hatchery design does not allow complete separation of clean areas, such as egg disinfection rooms and incubators and the less clean areas such as the hatching and chick collection rooms.

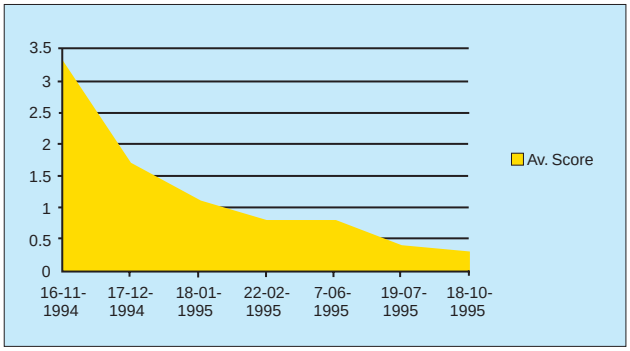
AT THE BROILER FARM:

- Litter material strongly contaminated with *Aspergillus*
- Feed contaminated (especially the rest feed from the previous crop remaining in the silos)

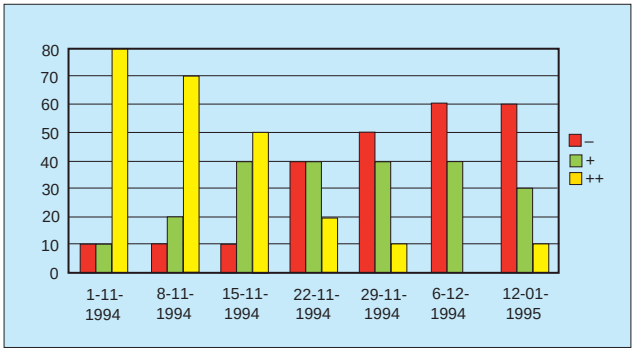
An aspergillosis prevention programme should certainly involve the use of Clinafarm spray and smoke in the standard hatchery cleaning and disinfection procedures. However, in certain cases with increased risk for *Aspergillus* infection pressure, Clinafarm can also be applied as a litter spray in the breeder farm or in the broiler farms. The product can also be used to disinfect contaminated feed silos or egg transportation trucks. Other useful applications are the regular disinfection of the ventilation channels in the hatchery with Cllinafarm smoke generator, or the disinfection of strongly contaminated eggs with the smoke or the spray.

Clinafarm fungicidal disinfectant offers the poultry industry a new, unique product that can be applied in a very versatile way to prevent problems with aspergillosis.

Evolution of the average Aspergillus-scores in a commercial hatchery in Belgium after the start of a Clinafarm disinfection programme.



Evolution of the % of Aspergillus positive air samples in a Venezuelian hatchery after the start of a Clinafarm disinfection programme.



Clinafarm TRADEMARK Smoke

- A ready for use smoke generator, containing 5g enilconazole
- After ignition, heavy smoke is generated for 20 to 40 seconds without a flame
- 5g active enilconazole is sufficient to disinfect a room of 50m^3
- Very well suited for the disinfection of closed rooms such as incubators, stables, feed silos, ventilation ducts etc

- The smoke is not toxic to humans or animals
- 1 day old chicks in smoke for 30 minutes show no signs of respiratory difficulties
- The smoke generator should be stored in a cool place (max 30°C)
- 3 year stability under normal storage conditions
- Place the generator on a heat resistant surface before ignition

- After ignition the wick burns into a powder causing an exothermic reaction, resulting in a heavy smoke production
- The enilconazole will be dispersed by the smoke in sub micron particle size
- After combustion, a charred grey black mass remains in the can. The total weight loss of the generator during smoking is around 12 grams

Clinafarm Smoke =
5g enilconazole generator

1 generator sufficient for 50m^3

The smoke should be contained for a few hours in closed rooms

Clinafarm TRADEMARK Spray

- Spray forms a stable micro-emulsion after dilution in water
- Has a high bioavailability and potent activity because of the low particle size of enilconazole
- Excipients are selected to improve the contact with surfaces, mycelium and spores
- Has oil based carrier and therefore will have a long residual effect

- Can be applied with a sprayer, nebuliser or thermal fogger
- Safe – no organic solvents, enilconazole is non toxic
- Clinafarm Spray is not corrosive
- Can be mixed with other detergents and disinfectants
- 5 year stability under normal storage conditions

EXAMPLE OF USE

- Prepare a ready to use emulsion (RTU) – dilute Clinafarm Spray 100 times with water
- Apply standard dose of 20mg enilconazole/m^2
- SPRAY: 1 litre RTU/75m^2
- FUMIGATE: (fogger/atomist) 1 litre RTU/300m^3
- When area is high in organic content (litter, nests etc) increase the dose x 2.5

To spray 1m^2 =
13.3 ml RTU solution or 0.13 ml Clinafarm Spray

1 litre Clinafarm treats 7500m^2

To nebulise 1m^3 =
3.3 ml RTU solution or 0.03 ml Clinafarm Spray

1 litre Clinafarm treats 30.000m^3