



Animal Reproduction
Received on: 03/08/2018
Accepted on: 20/05/2020

Reduction in the frequency of *Aspergillus* spp. in broiler facilities subjected to cleaning and disinfection

Redução na frequência de Aspergillus ssp. em instalações para frangos de corte submetidas à limpeza e desinfecção

BURBARELLI*¹, Maria Fernanda de Castro
<https://orcid.org/0000-0002-8079-2890>;

MORO², Maria Estela Gaglianone
<https://orcid.org/0000-0002-7630-169X>

LELIS¹, Karoline Deliberali
<https://orcid.org/0000-0003-3904-4273>

BORDIN³, Roberto de Andrade
<https://orcid.org/0000-0002-7067-7573>

GODOY², Sílvia Helena Seraphim de
<https://orcid.org/0000-0003-4652-1111>

FERNANDES², Andrezza Maria,
<https://orcid.org/0000-0001-6701-750X>

ALBUQUERQUE¹, Ricardo de
<https://orcid.org/0000-0002-5989-6740>

¹ Department of Animal Production and Nutrition - FMVZ/USP – Duque de Caxias Norte Av., 225. 13635-900 Jd. Elite Pirassununga – SP.

² Faculty of Animal Science and Food Engineering – FZEA-USP Duque de Caxias Norte Av., 225. 13635-900 Jd. Elite Pirassununga – SP

³ Nutrition, Animal Production, Health - FATEC-SP Carlos Baratinno street, nº 99 CEP 08773-600 Mogi das Cruzes – SP.

*Corresponding author e-mail: mfc@usp.com

ABSTRACT

The present study analyzes influence of two cleaning and disinfection protocols on frequency of *Aspergillus* spp. in broiler facilities. We conducted an observational study, applying two cleaning and disinfection protocols before housing 960 one-day-old broilers randomly allocated in 32 boxes with 30 birds. We considered two types of housing as independent experiments, which differed as to the bedding. First experiment consisted of new bedding materials, which we reused in second experiment. We applied two different treatments, Common treatment included sweeping organic matter, humidifying residual material with low pressure water, washing with water and neutral detergent, with posterior rinsing. European treatment was according to the standards of the manufacturer of disinfectants. Procedures included dry and humid organic matter removal, humidification, washing with high pressure water, application of detergent, rinsing, and application of two compound disinfectants: glutaraldehyde (250 g/L) + formaldehyde (185 g/L); p-chloro-m-cresol (210 g/L). To evaluate the presence of *Aspergillus* spp., we collected samples from surfaces and equipment before and after cleaning and disinfection,





at 7, 14, 28, and 42 days of experimental period. Mycological assessment included streaking on Petri dishes containing AFPA (*Aspergillus Flavus* and *Parasiticus* Agar, Sigma Aldrich®). Common cleaning and disinfection treatment led to a higher presence of fungus in floors and walls (83% vs. 0% $p = 0.0030$ and 50% vs. 0% $p = 0.0450$) in first and second experiments, respectively. As this study demonstrates by using the European procedures, performing a detailed cleaning and disinfection protocol reduces the presence of *Aspergillus* spp. in broiler facilities.

Keywords: Biosecurity, Hygiene, Mycology, Poultry Health

RESUMO

O presente estudo analisou a influência de dois protocolos de limpeza e desinfecção na frequência de *Aspergillus* spp. em instalações de frangos de corte. Realizou-se estudo observacional, aplicando-se dois protocolos de limpeza e desinfecção antes do alojamento de 960 pintainhos de um dia distribuídos aleatoriamente em 32 boxes com 30 aves. Foram realizados 2 experimentos independentes, que diferiam quanto ao material de cama, sendo nova no primeiro e reutilizada no segundo. Realizou-se dois tratamentos: Comum que incluiu varrição da matéria orgânica, umidificação do material residual com água sob baixa pressão, lavagem com detergente neutro e enxágue. O tratamento Europeu seguiu as recomendações do fabricante dos desinfetantes, incluindo remoção de matéria orgânica seca e úmida, umidificação, lavagem com água sob alta pressão, aplicação de detergente, enxágue e aplicação de dois desinfetantes compostos: glutaraldeído (250 g / L) + formaldeído (185 g / L); p-cloro-m-cresol (210 g / L). Foram coletadas amostras de superfícies e equipamentos antes e após a limpeza e desinfecção, aos 7, 14, 28 e 42 dias do período experimental para avaliação micológica, realizada em placas de Petri contendo AFPA (*Aspergillus Flavus* e *Parasiticus* Agar, Sigma Aldrich®). O tratamento Comum obteve maior presença de fungos nos pisos e paredes (83% vs. 0% $p = 0,0030$ e 50% vs. 0% $p = 0,0450$) no primeiro e segundo experimentos, respectivamente. Este estudo demonstra que a execução de um protocolo detalhado de limpeza e desinfecção, como o tratamento Europeu, reduz a presença de *Aspergillus* spp. em instalações de frangos de corte.

Palavras chave: Biosseguridade, Higiene, Micologia, Saúde aviária.





INTRODUCTION

Broiler facilities have ideal characteristics for the dispersion of fungi and their metabolites (Viegas et al., 2014; 2016). Dust particles can act as carriers, facilitating their dispersion and consequent inhalation by birds (Viegas et al., 2015). Bird exposure to *Aspergillus* spp. spores generally occurs after the introduction of contaminated bedding and feed (Kapetanov et al., 2015).

Studies on the presence of *Aspergillus* spp. in broiler breeders should consider the potential of these fungi to cause economic losses in production. Such losses can occur due to aspergillosis, a respiratory disease with high morbidity and mortality, which can affect all bird species, especially when young (Arné et al., 2011; Kapetanov et al., 2015).

Aspergillus spp. also has zoonotic potential, causing pulmonary infections in immunosuppressed populations and allergic reactions in immunocompetent ones. Moreover, *Aspergillus* spp. dispersion in the rural work environment, especially in poultry facilities, is an occupational health problem (Fairs et al., 2010; Chotirmall et al., 2013; Cafarchia et al., 2014).

Procedures for cleaning and disinfecting the facilities, as well as microbiological control of feed, are fundamental for reducing the risk of infections in birds and humans (Grezzi, 2007). These procedures must consider systematic methods. Despite the wide use of water alone for cleaning purposes, studies do not recommend this practice. This also applies to detergents, which although slightly germicidal, are not suitable as

disinfectants or sanitizers (Grezzi, 2007; Cafarchia et al., 2014; Kapetanov et al., 2015).

Thus, the present study evaluates the influence of two cleaning and disinfection protocols on the frequency of *Aspergillus* spp. in broiler facilities.

MATERIALS AND METHODS

The present study followed the ethical principles of animal experimentation and was approved by the Ethics Committee on Animal Use of the Faculty of Veterinary Medicine and Animal Science at the University of São Paulo, under Protocol 2029/2010.

To evaluate the efficiency in eliminating strains of the fungus *Aspergillus* spp. in broiler facilities, we conducted an observational study consisting of two cleaning and disinfection protocols before housing 960 one-day-old broilers, which we randomly allocated in 32 boxes with 30 birds each. We raised the birds in these conditions until 42 days of age.

We used two types of housing in a conventional shed divided into boxes (2.2 x 1.2 m). These two experiments differed only as to the bedding. The first type of housing included new bedding materials, namely pine wood shavings, which were previously fumigated. The bedding used in the second experiment came from the first experiment and was previously windrowed for anaerobic fermentation.

The diet of the animals consisted of corn and soybean meal, being the same for all birds in both housings, meeting the requirements proposed by Rostagno et al. (2011).





We conducted two cleaning and disinfection protocols, applying each of them in 16 experimental boxes of the facilities previously to housing. We applied the protocols to all surfaces and equipment used for broiler breeding, such as: floors, walls, curtains, roofs, screens, feeders, drinkers, operator's boots, bell jars, lamps, shovels, brooms, and buckets.

The so-called "common" protocol consisted of simple cleaning and disinfection procedures: sweeping of floors and dry removal of organic matter, wet removal of organic matter with low pressure water, washing with neutral detergent, rinsing, and drying at room temperature.

The so-called "European" protocol consisted of detailed cleaning and disinfection procedures, with the concentrations and action times following the manufacturer's guidelines. For that purpose, we performed the following steps: dry removal of organic matter by sweeping; humidification of the environment with low pressure water - application with a hose connected to a conventional tap; washing with water using a jet washer (pressure of 150-180 bar); application of alkaline detergent diluted in water at a concentration of 4%, at low pressure, with 20 to 30 minutes of action; rinsing of the premises with water (pressure of 150-180 bar); drying of the environment; application of disinfectant composed of glutaraldehyde (250 g/L) and formaldehyde (185 g/L) at a concentration of 0.5%, at low pressure, with a backpack sprayer, on all surfaces of the shed and objects; application of disinfectant composed of p-chloro-m-cresol (210 g/L) at a concentration of

4%, at low pressure, with a backpack sprayer, only on the floor and walls up to 0.5 m high.

To assess the presence of *Aspergillus* spp., we collected samples before and after cleaning and disinfection, on the 7th, 14th, 28th, and 42nd days of the experimental period. We analyzed floors, walls, feeders, drinkers, and curtains. For floors, walls, and feeders, we collected three samples per treatment. To analyze the surfaces, we used swabs in an area of 50 cm² according to the methodology proposed by Evancho et al. (2001). We rubbed the surfaces with sterile swabs soaked in 0.5% sterile peptone water. After this procedure, we placed the swabs in test tubes containing 10 mL of 0.5% peptone water, which we immediately transported to the laboratory for dilution and incubation in Petri dishes. We performed successive dilutions up to 10⁻³.

For homogeneous distribution, we used a Drigalski spatula for 100 µL of the dilutions in Petri dishes containing about 15 mL of melted and cooled AFPA (*Aspergillus Flavus* and *Parasiticus* Agar, Sigma Aldrich®). After streaking, we incubated the plates at room temperature for five days (Pitt et al., 1983).

We selected the plates that showed growth of fungal colonies with characteristics consistent with *Aspergillus* spp. (white surface that turns yellow, green, brown, or black; velvety texture; white, golden, or brown on reverse). Then, we subjected these plates for microculture on potato dextrose agar (Sigma Aldrich®), and stained them with methylene blue. We analyzed the samples microscopically, considering





those with septate hyphae (2.5 - 8.0 μm in diameter), aspergillary heads with vesicles at the end of the conidiophores and elongated external spores (phialides) as positive for *Aspergillus* spp. (Larone, 2000).

We performed nonparametric tests for statistical analysis of the data since these did not meet the assumptions of homogeneity of the variances and normality of T residues. Furthermore, we compared qualitative results (presence/absence) using the Chi-square test at 5% significance, submitting the frequencies obtained to the FREQ procedure of the SAS (SAS, 2012).

RESULTS AND DISCUSSION

Table 1 shows the results of the *Aspergillus* frequency in the samples collected in the two experiments. The first experiment showed a difference in the frequency of *Aspergillus* only on floors after cleaning and disinfection. The floors subjected to common treatment had the highest frequency of *Aspergillus* contamination.

The second experiment showed a difference in the occurrence of *Aspergillus* in the samples of walls after cleaning and disinfection, and in the samples of curtains at 7 days after cleaning and disinfection. For both, the highest fungal prevalence occurred when performing the common protocol.





Table 1: Frequency of *Aspergillus* spp. in samples from the observational study of “European” and “common” cleaning and disinfection protocols. In experiment 1, the birds' bedding consisted of new pine wood shavings. In experiment 2, these pine wood shavings were reused.

	Experiment 1			Experiment 2		
	European	Common	P*	European	Common	P*
Floor						
Before	16.66% (1/6)	0% (0/6)	0.296	16.66% (1/6)	50% (3/6)	0.221
After	0% (0/6)	83.33% (5/6)	0.003	16.66% (1/6)	16.66% (1/6)	1.000
7 days	0% (0/6)	0% (0/6)	1.000	50% (3/6)	100% (6/6)	0.045
28 days	0% (0/6)	33.33% (2/6)	0.121	0% (0/6)	16.66% (1/6)	0.296
42 days	0% (0/6)	0% (0/6)	1.000	33.33% (2/6)	16.66% (1/6)	0.416
Wall						
Before	0% (0/6)	0% (0/6)	1.000	0% (0/6)	0% (0/6)	1.000
After	16.66% (1/6)	0% (0/6)	1.000	0% (0/6)	50% (3/6)	0.045
7 days	0% (0/6)	0% (0/6)	1.000	50% (3/6)	33.33% (2/6)	0.558
28 days	16.66% (1/6)	16.66% (1/6)	1.000	0% (0/6)	0% (0/6)	1.000
42 days	0% (0/6)	0% (0/6)	1.000	16.66% (1/6)	0% (0/6)	0.296
Feeder						
Before	33.33% (2/6)	33.33% (2/6)	1.000	16.66% (1/6)	50% (3/6)	0.221
After	0% (0/6)	0% (0/6)	1.000	50% (3/6)	33.33% (2/6)	0.558
7 days	16.66% (1/6)	0% (0/6)	0.296	50% (3/6)	33.33% (2/6)	0.558
28 days	0% (0/6)	0% (0/6)	1.000	0% (0/6)	33.33% (2/6)	0.121
42 days	0% (0/6)	0% (0/6)	1.000	0% (0/6)	0% (0/6)	1.000
Drinker						
Before	33.33% (2/6)	33.33% (2/6)	1.000	16.66% (1/6)	0% (0/6)	0.296
After	16.66% (1/6)	0% (0/6)	0.296	33.33% (2/6)	16.66% (1/6)	0.505
7 days	0% (0/6)	0% (0/6)	1.000	16.66% (1/6)	16.66% (1/6)	1.000
28 days	0% (0/6)	0% (0/6)	1.000	0% (0/6)	16.66% (1/6)	0.296
42 days	0% (0/6)	16.66% (1/6)	0.296	16.66% (1/6)	16.66% (1/6)	1.000
Curtain						
Before	0% (0/4)	25% (1/4)	0.285	25% (1/4)	25% (1/4)	1.000
After	0% (0/4)	0% (0/4)	1.000	0% (0/4)	0% (0/4)	1.000
7 days	0% (0/4)	0% (0/4)	1.000	25% (1/4)	100% (4/4)	0.028
28 days	0% (0/4)	0% (0/4)	1.000	0% (0/4)	0% (0/4)	1.000
42 days	0% (0/4)	25% (1/4)	0.285	25% (1/4)	25% (1/4)	1.000

* Chi-square test at 5% significance ($P < 0.05$)





The type of bedding used did not influence the results obtained in the present study.

The findings corroborate Kapetanov et al. (2015), who studied the presence of *Aspergillus* in sheds for broilers and turkeys, in beddings and embryonated eggs, using surface swabs. The authors found a 36% frequency for the evaluated surfaces, highlighting the need for sanitary control in reducing the frequency of *Aspergillus*.

Viegas et al. (2011) found *Aspergillus* in 7% of the air samples and in 5% of the analyzed surfaces. These frequencies are lower than those of the present study; even so, the authors emphasize the importance of identifying *Aspergillus* in broiler breeding due to the negative consequences to the performance and welfare of the animals (Andreatti Filho, 2000).

The wide worldwide distribution of *Aspergillus* in sheds and hatcheries is demonstrated in the findings of Mustafa (2017), who found high frequencies in Iraq; Lorin et al. (2017), in Romania; and Viegas et al. (2017), in Portugal. Thus, attention should be paid to the health implications from the presence of these fungi in broiler sheds, with the possibility of contamination of animals and development of aspergillosis; contamination of carcasses with mycotoxins; and occupational diseases with the exposure of workers to fungi and mycotoxins (Viegas et al., 2011).

The type of ventilation, air humidity, and ambient temperature (Sajid et al., 2006) can influence the occurrence of *Aspergillus* in poultry facilities. Therefore, it is important to control the microclimate of the facilities so as to

reduce the development of these fungi (Cafarchia et al., 2014; Kapetanov et al., 2015). Conventional poultry facilities, as in the present study, have less microclimate control, mainly regarding ventilation, which can favor dispersion and contamination by *Aspergillus* (Baracho et al., 2001). It is thus fundamental to adopt procedures that reduce the fungal load of the environment, such as cleaning the facilities.

Glutaraldehyde and formaldehyde are compounds with good efficiency in eliminating fungi (Peterson et al., 2008) in poultry facilities. These compounds are present in the disinfectants used in the European cleaning and disinfection protocol. Such composition may relate to the lower fungal prevalence in the first and second experiments of the present study. Moreover, the organic matter present in poultry environments acts as a barrier to the action of disinfectants. In this way, procedures such as washing with high pressure water help in its removal, allowing greater efficiency of disinfectants (Peterson et al., 2008). In the common protocol, the absence of an efficient step to remove organic matter may relate to the higher frequencies of *Aspergillus*.

Cleaning and disinfection procedures such as those performed in the European system are fundamental actions in the prevention of aerial dispersion and contamination by *Aspergillus* (Rajmani et al., 2011; Cafarchia et al., 2014). Adding to this evidence, the present study demonstrated that performing a simple cleaning and disinfection protocol has little efficiency in reducing the fungal contamination of broiler





facilities. The implementation of the European protocol, which consists of sequential practices for the use of detergent and disinfectants with a wide spectrum of action, reached greater efficiency in reducing the frequency of *Aspergillus*.

Using a cleaning and disinfection program with detailed steps, such as the European protocol, leads to greater efficiency in reducing the occurrence of *Aspergillus* spp. in broiler facilities.

Acknowledgements

Research funded by the São Paulo State Research Support Foundation – FAPESP, Process No. 2010/04474-9.

REFERENCES

ARNÉ, P.; THIERRY, S.; WANG, D.; DEVILLE, M.; LOC'H, L.; DESOUTTER, A.; GUILLOT, J. *Aspergillus fumigatus* in poultry. **International journal of microbiology**, v.2011, 2011.

ANDREATTI FILHO, R.L. *Enfermidades micóticas*. In: BERCHIERI JÚNIOR., A. & MACARI, M. (Ed.) **Doenças das aves**. Campinas: FACTA. p.369-378, 2000.

BARACHO, M. S.; NÄÄS, I.; MIRAGLIOTTA, M. Evaluation of broiler housing indoor air quality during heating and during the use of two types of ventilation: natural and tunnel. In livestock environment VI: **Proceedings of the Sixth International Symposium**, Galt House Hotel, Louisville, Kentucky, p. 377, 2001.

CAFARCHIA, C.; CAMARDA, A.; IATTA, R.; DANESI, P.; FAVUZZI, V.; DI PAOLA, G.; PUGLIESI, N.; CAROLI, A.; MONTAGNA, M. T.; OTRANTO, D. Environmental contamination by *Aspergillus* spp. in laying hen farms and associated health risks for farm workers. **Journal of Medical Microbiology**, v.63, n.3, p.464-470, 2014.

CHOTIRMALL, S. H.; AL-ALAWI, M.; MIRKOVIC, B.; LAVELLE, G.; LOGAN, P. M.; GREENE, C. M.; MCELVANEY, N. G. *Aspergillus*-associated airway disease, inflammation, and the innate immune response. **Biomed Research International**, v. 2013, p.1-14, 2013.

EVANCHO, G.M.; SVEUM, W.H.; MOBERG, L.J.; FRANK, J.F. **Compendium methods for the microbiological examination of foods** (4ed.), Iowa, American Public Health Association, p. 25–35, 2001.

FAIRS, A.; WARDLAW, A. J.; THOMPSON, J. R.; PASHLEY, C. H. Guidelines on ambient intramural airborne fungal spores. **Journal Investigation Allergology Clinical Immunology**, v.20, p.490–498, 2010.

GREZZI, G. Limpeza e desinfecção na avicultura. In: Conferência Apinco, Santos. **Anais**. Santos: FACTA, 2007

KAPETANOV, M.; LJUBOJEVIĆ, D.; STOJANOV, I.; ŽIVKOV-BALOŠ, M.; PELIĆ, M.; PAJIĆ, M. The prevalence of aspergillosis in poultry and control





measures-our experience. In "One Health-New Challenges", First International Symposium of Veterinary Medicine, Vrdnik, Serbia. **Proceedings**, p. 97-104, 2015.

LARONE, D.H. **Medically Important Fungi: A guide to Identification**, 3^o ed., Washington, DC., 2000

LORIN, D.; TEUȘDEA, V.; MITRĂNESCU, E.; DANEȘ, M.; ȘTEF, L.; MOȘNEANG, C. L.; Cristina, R. T. (2017). The mycobiota composition in eight Romanian representative poultry and swine farms. **Romanian Biotechnological Letters**, v.22, n.5, p.12961-12971, 2017.

MENDES, A. A; NASS, I. A; MACARI, M; **Produção de Frangos de Corte**. Campinas, FACTA, 2004.

MUSTAFA, S. O. (2017). The Effect of Aspergillus Fungi, Other Fungus, and Isolated Salmonella and E. coli Bacteria on Poultry Farms and Poultry Hatcheries at Veterinary Laboratory in Veterinary Directorate in Duhok, Kurdistan Region of Iraq. **EC Microbiology**, v.5, p. 52-58, 2017.

PETERSON, C. A.; DVORAK, G.; SPICKLER, A. R. Maddie's infection control manual for animal shelters: for veterinary personel. **Pet Rescue Foundation**, C. 101, p. 42-64, 2008.

PITT, J.I; HOCKING, A; GLEND, D. An Improved Medium for the Detection of Aspergillus flavus and A. parasiticus. **Journal of Applied Bacteriology**, v.54, 109-114, 1983.

RAJMANI, R.S.; SINGH, A.P.; SINGH, P.K.; DOLEY, J.; VERMA, S.P. Fungal Contamination in Eggs. **Journal of Veterinary Public Health**, v. 9, 59-61, 2011.

ROSTAGNO, H. S.; ALBINO, L. F. T.; DONZELE, J. L. ; GOMES, P. C.; OLIVEIRA, R. F.; DLOPES, . C.; FERREIRA, A. S.; BARRETO, S. L. T. **Tabelas brasileiras para aves e suínos Composição de alimentos e exigências nutricionais**. 3rd ver. ed. Editora UFV. Viçosa, MG, Brazil, 2011.

SAS Institute. **User's guide**. Statistics Version 9.2 ed. Cary: SAS Inst, Inc., Cary, NC, 2012.

SAJID M. A.; KHAN I. A.; RAUF U. Aspergillus fumigatus in commercial poultry flocks, a serious threat to poultry industry in Pakistan. **Journal Animal and Plant Science**, v.16, n. 3/4, p. 79-81, 2006.

VIEGAS, C.; VIEGAS, S.; VERÍSSIMO, C.; ROSADO, L.; SANTOS, C. S. Possíveis implicações da contaminação fúngicas num aviário, **Saúde & Tecnologia**, n.6, p.17-23, 2011.

VIEGAS, C.; FARIA, T.; CAETANO, L. A.; CAROLINO, E.; GOMES, A. Q.; VIEGAS, S. Aspergillus spp. prevalence in different Portuguese occupational environments: What is the real scenario in high load settings?. **Journal of Occupational and Environmental Hygiene**, v.14, n.10, p.771-785, 2017.





VIEGAS, C.; FARIA, T.; GOMES, A.;
SABINO, R.; SECO, A.; VIEGAS, S.
FUNGAL contamination in two
Portuguese wastewater treatment plants.
**Journal Toxicology Environmental
Health**, n.77, v.1–3, p.90–102, 2014.

VIEGAS, C.; SABINO, R.; BOTELHO,
D.; SANTOS, M.; GOMES, A. Q.
Assessment of exposure to the
Penicillium glabrum complex in cork
industry using complementing methods.
**Archives Industrial Hygiene and
Toxicology** n.66, v.3, p.203–207, 2015.

VIEGAS, C.; FARIA, T.; MENESES,
M.; CAROLINO, E.; VIEGAS, S.;
GOMES, A. Q.; SABINO, R. Analysis
of surfaces for characterization of
fungal burden – does it matter?
**International Journal of
Occupational Medicine and
Environmental Health**, n.29, v.4,
p.623-632, 2016.

