

Respiratory Mycosis in Commercial Pullets in the Peninsula of Yucatan, Mexico



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Summary

The species *Aspergillus* spp. Is the main cause of respiratory mycosis with high mortality and morbidity, reducing the poultry farmer's profits. The objective of this study was to isolate and identify the presence of the main fungal strains in a commercial farm of replacement pullets. 60 pullets were sampled from the reception in the farm (day one) for three weeks (180 birds) and 15 of those housed in the shed at day eight of age (180 birds). A 1cm portion of lung was seeded on potato dextrose agar. The fungi were identified by morphometry. The data was analyzed by SPSS for Windows. They isolated and identified *Aspergillus fumigatus*, *A. flavus*, *A. niger* and the presence of *Zygomycetes* spp. ($P>0.05$). Mortality due to aspergillosis occupied the second place ($P<0.01$). It is concluded that in addition to aspergillosis zygomycosis is the cause of respiratory problems and mortality in farms.

Keywords: Aspergillosis; Mortality; Zygomycosis; Replacement pullets

Introduction

Aspergillosis is a condition produced by the fungus of the genus *Aspergillus*, mainly belongs to the storage flora, which is widely distributed in nature; its members are the cause of opportunistic infections that affect any captive or free, domesticated or wild bird [1-3]. Infection with *Aspergillus fumigatus* is a common disease that causes a respiratory condition known as respiratory mycosis or pulmonary aspergillosis when animals are exposed to high concentrations of airborne conidia that invade the lungs [4,5].

Mycosis occurs clinically in two forms: acute aspergillosis characterized by acute outbreaks in young birds with high morbidity and mortality, no signs are observed, except suffocation occasionally a "whistle" and diarrheas are observed, the first lesions to the necropsy are located in the air sacs and lungs; and subacute or chronic aspergillosis observed in breeders and sometimes in adult flocks or aviaries, is characterized by dyspnea, accelerated and fatigued breathing, progressive weight loss with cachexia and sometimes kerato conjunctivitis [5-7].

Beds, food, incubators, hatchers, and transport equipment with wet surfaces contaminated with the fungus are the main cause of the problem's spread; poor nutrition, lack of hygiene and stress are predisposing factors; spores can penetrate the egg shell and infect embryos that already sprout with lesions [8].

The aviculturists keep records of the infectious and non-infectious problems of flocks due to the economic impact they

represent; However, many times they do not quantify the importance of each one of the farm conditions individually. Hence, the objective of the present study was the sampling of replacement pullets of one and eight days of age to describe the abundant species of fungi that can be isolated and identified by morphometry and the impact on mortality that they represent in the farm.

Materials and Methods

The data was obtained from a commercial farm of replacement pullets of the Bovans White genetic line, housed in a floor, located in the state of Yucatan, Mexico, 20° 57 '6.82 "N and 89° 56' 30.69" W at a height 5m. The climate is of the Aw0 type, the driest of the hot climates, sub-humid climates according to the Koppen classification modified by García [9], with an annual temperature between 21.0 and 33.8 °C [10]. One-day-old pullets were randomly sampled from each batch of incubator on arrival at farm (reception) for a total of 30 pullets per sample. The receptions were two days a week with an average of 52,000 pullets received per week. Samples were taken during three consecutive weeks. At eight days of age, 15 pellets housed in booth were randomly sampled, 12 booths with a population of 12,000 birds per booth, for a total of 360 pullets (180 on day one and 180 on day eight). Pullets were sacrificed by cervical dislocation based on NOM-033-ZOO-1995. A fragment of a 1cm lung was seeded using Petri dishes with potato Dextrose agar, incubated for five days at room temperature and the macroscopic characteristics of the colonies were observed. A sample was taken

from the colony and stained with cotton blue for morphometrics identification using the Department of Pathology database at the University of Texas Health Science Center San Antonio, TX.

Data were analyzed using SPSS for Windows using a contingency table for age at sampling and week; and frequencies between causes of mortality, chi square test ($P < 0.05$) [11].

Results and Discussion

The results found during the sampling at day one of age in pullets are presented in Table 1. During the sampling, the presence of *Aspergillus flavus* (12.8%), *A. fumigatus* (14.4%), *A. niger* (13.3%) was identified by morphometry. (%), and in 18 observations, *A. flavus* plus zygomycota (10.0%) were isolated and identified in 18

observations. *A. niger* plus zygomycete (10.0%) (Figure 1), which were isolated. No difference was found ($P > 0.05$) with respect to the frequency of fungi isolated by sampling. Larone [12] mentions that *A. fumigatus* is the most commonly isolated species in nature due to its unique ability to grow at temperatures between 20 to 50 °C and rapid growth, followed by *A. flavus* and *A. niger* and have returned the main pollutants in places conducive to their growth. The study showed that replacement pullets can get infected with *Aspergillus* to farm and find in the same bird the presence of two different species of *Aspergillus* from one day of age. This supports the fact that embryos may be contaminated during incubation due to storage and inadequate disinfection of incubation facilities and equipment [8].

Table 1: Week contingency table for fungus in pullets sampled at day one of age at reception on farm.

Fungus								
Week		<i>A. flavus</i>	<i>A. flavus</i> + zygo*	<i>A. fumigatus</i>	<i>A. niger</i>	<i>A. niger</i> + zygo*	Negative	Total
1	Count	6	7	8	6	6	27	60
	% of week	10.00%	11.70%	13.30%	10.00%	10.00%	45.00%	100.00%
	% of Fungus	26.10%	38.90%	30.80%	25.00%	33.30%	38.00%	33.30%
2	% of total	3.30%	3.90%	4.40%	3.30%	3.30%	15.00%	33.30%
	Count	9	7	9	10	6	19	60
	% of week	15.00%	11.70%	15.00%	16.70%	10.00%	31.70%	100.00%
	% of Fungus	39.10%	38.90%	34.60%	41.70%	33.30%	26.80%	33.30%
	% of total	5.00%	3.90%	5.00%	5.60%	3.30%	10.60%	33.30%
3	Count	8	4	9	8	6	25	60
	% of week	13.30%	6.70%	15.00%	13.30%	10.00%	41.70%	100.00%
	% of Fungus	34.80%	22.20%	34.60%	33.30%	33.30%	35.20%	33.30%
	% of total	4.40%	2.20%	5.00%	4.40%	3.30%	13.90%	33.30%
Total	Count	23	18	26	24	18	71	180
	% of week	12.80%	10.00%	14.40%	13.30%	10.00%	39.40%	100.00%
	% of Fungus	100.00%	100.00%	100.00%	100.00%	100.00%	100.00%	100.00%
	% of total	12.80%	10.00%	14.40%	13.30%	10.00%	39.40%	100.00%

*Zygomycetes

No differences between chi-square means ($P > 0.05$)

The results of the sampling at the eighth day of age are observed in Table 2, it can be seen that the presence of *zygomycetes* (n=22) was the most abundant compared to the presence of *A. flavus* (n=1) and *A. niger* (n=1) No difference was found (P>0.05) with respect to the frequency of fungi isolated by sampling. The presence of

Zygomycetes spp. in pullets, they are considered more aggressive due to their rapid growth (three days) and tissue invasion (Larone, 1995), because they can grow on the floor at a temperature between 50 to 55 °C, humidity between 60 to 85%.

Table 2: Weekly contingency table for fungus in pullets sampled at day eight of age in the farm.

		Fungus				
Week		A. flavus	A. niger	Zygomycetes	Negative	Total
1	Count	0	0	9	51	60
	% of week	0.00%	0.00%	15.00%	85.00%	100.00%
	% of Fungus	0.00%	0.00%	40.90%	32.70%	33.30%
	% of total	0.00%	0.00%	5.00%	28.30%	33.30%
2	Count	0	0	7	53	60
	% of week	0.00%	0.00%	11.70%	88.30%	100.00%
	% of Fungus	0.00%	0.00%	31.80%	34.00%	33.30%
	% of total	0.00%	0.00%	3.90%	29.40%	33.30%
3	Count	1	1	6	52	60
	% of week	1.70%	1.70%	10.00%	86.70%	100.00%
	% of Fungus	100.00%	100.00%	27.30%	33.30%	33.30%
	% of total	0.60%	0.60%	3.30%	28.90%	33.30%
Total	Count	1	1	22	156	180
	% of week	0.60%	0.60%	12.20%	86.70%	100.00%
	% of Fungus	100.00%	100.00%	100.00%	100.00%	100.00%
	% of total	0.60%	0.60%	12.20%	86.70%	100.00%

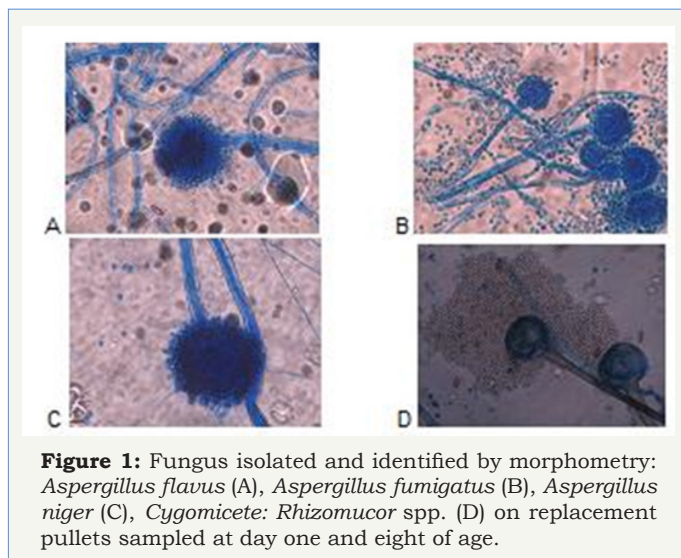


Figure 1: Fungus isolated and identified by morphometry: *Aspergillus flavus* (A), *Aspergillus fumigatus* (B), *Aspergillus niger* (C), *Cygomicete: Rhizomucor* spp. (D) on replacement pullets sampled at day one and eight of age.

Sampling at eight days of age showed that the presence of *Zygomycetes* spp. (12.2%), was greater than the presence of the genus *Aspergillus* spp. (0.6%), due to the invasion capacity of the first [12]. The appearance of problem by aspergillosis was observed from the fourth day of age in the pullets housed in the farm and persisted until 14 days of age.

The lack of an adequate disinfection or a role of products in an incubator can lead to the presence of fungi in the facilities and transport equipment, putting the pullets at risk, as shown by the

results found on day one and during its first 14 days of life where mortality by aspergillosis represented 0.99% of accumulated mortality, if you take into account that the accumulative mortality of the genetic line is 3.0% at 112 days.

Conclusion

The presence of *Aspergillus* spp. and *Zygomycetes* spp. was demonstrated in the same bird. The presence of fungi of the genus *Zygomycetes* in pullets with respiratory problems, are the cause of the infection that should be referred to as zygomycosis, which together with aspergillosis, are causing respiratory problems that increase the mortality of replacement pullets on farms.

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