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FUNGAL SPECIES ISOLATED FROM UNHATCHED HEN EGGS

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Abstract

This study investigated the fungal load in unhatched eggs as well as the presence and proportion of fungal species known to contaminate eggs and chicken embryos. The hatching percentage of three series of 60 chicken eggs subjected to incubation was very low. 136 (n=180) samples collected from unhatched chicken eggs, used in the incubation of 3 series consisting of 60 fresh eggs, were analyzed. For primary inoculation and colony isolation, the decimal dilution technique was used. From the last dilution, inoculations were made using Potato-Dextrose-Agar (PDA) medium. After incubation at 25°C for 7 days, each colony was reinoculated to obtain pure cultures, which were characterized macroscopically and microscopically in order to identify the species of microscopic fungi. The proportion of isolated fungal species was different in the three series of unhatched eggs, but in all of them the *Aspergillus* genus prevailed (44%, series 1, 43.7%, series 2 and 37.1%, series 3). Other fungal species in a large proportion belonged to the genus *Penicillium* (20%, 15.6%, 29.6%) and *Fusarium* (12%, 28.1%, 29.6%). The species with pathogenic potential, most frequently identified according to macroscopic and microscopic characteristics were: *Aspergillus niger* (13/35), *Aspergillus fumigatus* (12/25), *Aspergillus flavus* (10/35) and *Penicillium crysogenum* (10/18) and *Fusarium oxysporum* (12/20).

Introduction

So far, So far, the issue of mycobiota has been studied mostly on table eggs and less on hatching eggs, as research has focused on other factors that influence embryogenesis and especially hatching. The latest studies highlight a threat caused by contamination of the eggshell with microscopic fungi (5, 13). Incubation conditions (high air humidity and diffusion of vapors from the contents of the eggs) favor the development of microscopic fungi on the eggshells. Consequently, the embryos may be contaminated with mycelia and die (4, 6). Egg production conditions such as quality of straw and feed, presence of dust, temperature and humidity can cause fungal contamination of eggshells. It is known that eggs that are produced inside hens kept on litter are most susceptible to contamination by microscopic fungi (18). Contamination in indoor, permanent bedding systems is usually caused by wet bedding and dirty nests (14, 19). Researchers examined different types of litter used in poultry farming and found that they contained genera of fungi such as *Aspergillus*, *Cladosporium*, *Drechslera*, *Penicillium*, *Stemphylium* and *Fusarium* (17, 22). The fungal load (CFU/g) in the litter was the highest (28.49 log CFU/g) in straw chaff, being considered to have the highest risk of contamination for eggs. In addition, in this type of litter, a high concentration of ergosterol, a chemical compound present in the fungal wall, of 605.74 mg/kg was demonstrated, which means that this would be a good chemical indicator of microscopic fungal contamination of the litter material (22). The spores present in the air can also cause numerous diseases (10, 20). The hatchability of the fertile egg of hens can be adversely affected by factors such as temperature, humidity, ventilation, sanitary management of birds and inadequate facilities as well as bacterial and fungal infections [15]. Rodríguez-Moya *et al.*, 2017 (15), state that the ideal temperature for good embryo development is between 37 and 38 degrees Celsius. If the embryos are exposed to 47 degrees Celsius, they die immediately. Embryos, on the other hand, can withstand low temperatures for a short period of time, but cold reduces the yolk consumption of embryos, which prevents them from surviving (7, 16). Mortality and embryonic development are significantly affected by the proportion of humidity inside the incubators. Van der Pole *et al.*, 2013, (24), found that a relative humidity of 30 to 35 percent increased embryonic mortality. It has also been shown that a low relative humidity in the incubator increases the evaporation of water from the eggs, which leads to embryonic dehydration and as a result, the live chick weight is lower compared to the egg weight (12). In addition, bacteria and fungi help reduce hatching. During incubation, both pathogens enter the egg through the pores of the eggshell. One of the most widespread bacteria in eggshells is *Salmonella spp.* (21). *Escherichia coli* is another species of pathogenic bacteria that causes embryonic death, if it enters the egg, passing through the pores of the eggshell (9). Fungi such as *Aspergillus fumigatus*, *Fusarium culmorum* and *Fusarium equiseti* could penetrate in eggs and contaminate the embryos, developing the mycelium that leads to embryonic death (23).

Knowing these aspects, the research carried out in this study sought to highlight the fungal load in unhatched eggs as well as the presence and proportion of fungal species known to contaminate eggs and chicken embryos (*Aspergillus*, *Penicillium*, *Fusarium*, *Alternaria*, *Cladosporium*).

Material and method

To carry out the study, the samples collected from the unhatched eggs obtained from the incubation of 3 series consisting of 60 fresh eggs from a farm where hens are raised for reproduction, the Ross308 hybrid, were analyzed. Their maintenance is done on the ground, with litter. The eggs are laid in nests equipped with a sloping floor and a place for collecting the egg. The eggs received for the purpose of the experiment were subjected to fumigation, on the farm, with the Fumagri product, in a hermetically sealed room, with fumigants, for 1 hour. They were transported for the experiment, in egg formwork, at a temperature of 20°C.

The eggs were incubated in an individual system, using a Cleo 5 incubator, at a temperature of 37.8 - 38.4°C and a relative air humidity of 55-65% during the incubation period, and in the last 3 days of 65-75% (fig. 1). The eggs were turned twice a day, daily, from day 4 to day 18 inclusive. In addition, at exact time intervals (5 days, 12 days, and 19 days) the mirage of the eggs was realized in order to assess the viability of the embryos. On day 21, the hatched chicks were counted, the hatching percentage was calculated, and the unhatched eggs were subjected to mycological examination. For the mycological examination, samples were collected from the inside of the 136 (n=180) unhatched eggs, in the microbiological niche. From each sample, decimal dilutions were made in order to obtain a lower concentration of fungi to ensure the development of isolated colonies. From the last dilution (x106), 1 ml was seeded each in two sterile Petri plates, after which 10 ml of culture medium, agar with potato extract and dextrose (PDA). Incubation was realised at 25 °C, for 7 days, to reach the maturity of the colonies. To identify the genus and species of fungi developed after seeding the samples with contents from unhatched eggs, primary examination of the macroscopic and microscopic characters of each colony grown on the seeded plates was made. Then secondary seeding was done to obtain isolated colonies (subculturing) and final examination of morpho-structural characters (2, 11).

Results and discussions

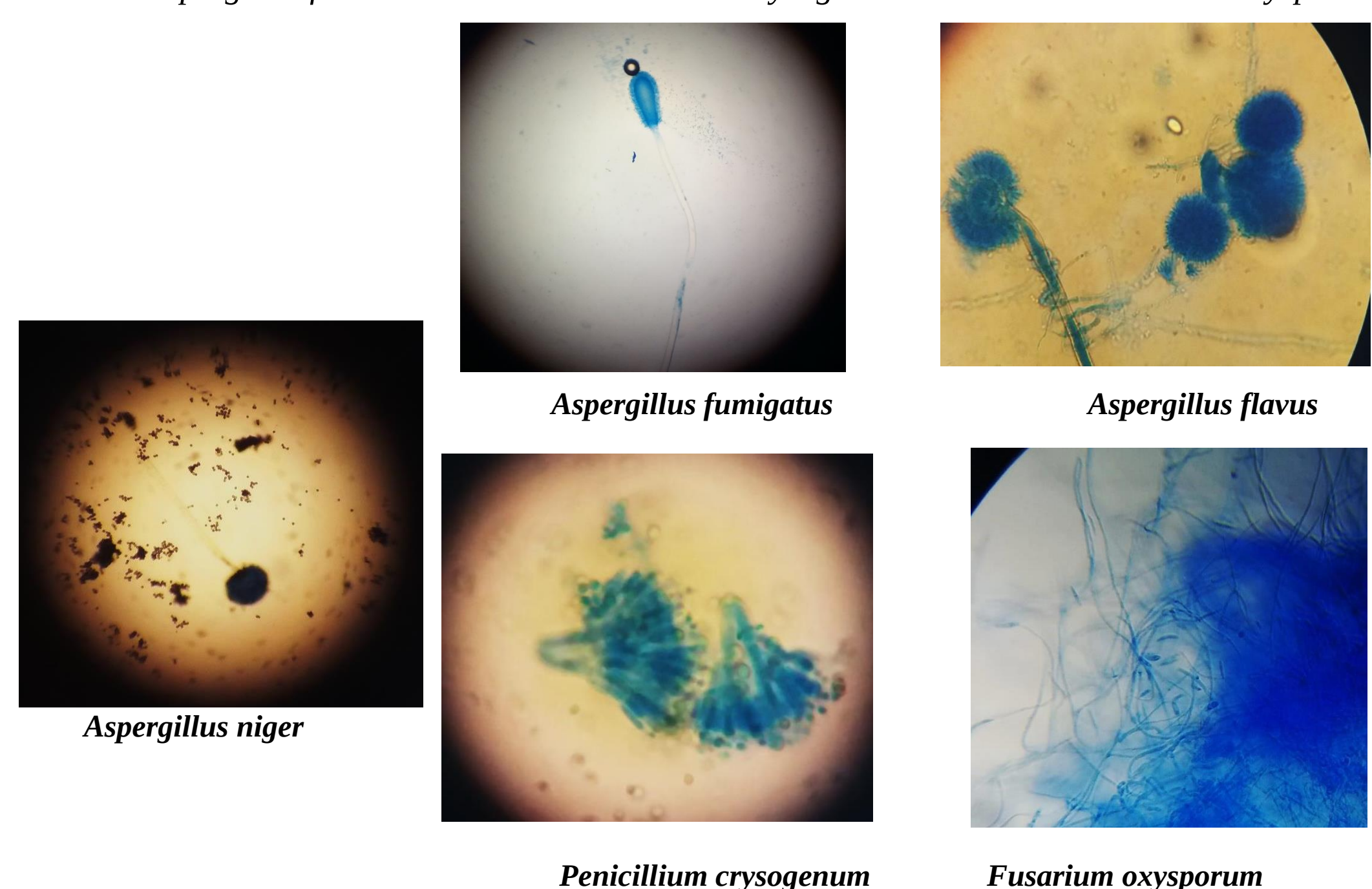
From the data obtained, it was found that after 5 days of incubation, the percentage of viable eggs was over 90%, in all three series of chickens, with the specification that in the second series, a lower percentage was found, of 93.3 compared to the third series, where the percentage was 95 and compared to series one, where the percentage of fertile eggs was the best, 96.6. After 12 days of incubation, the proportion of viable eggs dropped drastically in all three egg series by about 1.6-fold. Thus, in series one, only 60% of the eggs were viable, in series two, only 56.6%, and in series three, the percentage was 58.3. After 19 days of incubation, the percentage of viable eggs was 3.5 times lower, so that in series one only 28.3% of eggs were viable, in series two, only 25%, and in series three only 28.3%.

Hatched Egg Series	The genus or species of fungi isolated	Number of developed colonies	Proportion of total colonies (%)
Series 1 (number of colonies=25)	<i>Alternaria</i>	2	8
	<i>Aspergillus</i>	11	44
	<i>Cladosporium</i>	7	28
	<i>Penicillium</i>	5	20
	<i>Fusarium</i>	3	12
Series 2 (no. colonies=32)	<i>Alternaria</i>	1	3.1
	<i>Aspergillus</i>	14	43.7
	<i>Cladosporium</i>	3	9.3
	<i>Penicillium</i>	5	15.6
	<i>Fusarium</i>	9	28.1
Series 3 (number of colonies=27)	<i>Alternaria</i>	0	0
	<i>Aspergillus</i>	10	37.1
	<i>Cladosporium</i>	1	3.7
	<i>Penicillium</i>	8	29.6
	<i>Fusarium</i>	8	29.6

The genus of fungi isolated from unhatched eggs and their proportion

The number of chicks hatched from each series was very low, resulting in a hatch percentage of 25% in the first series, 23.3% in the second series and 25% in the third series. A total of 136 (n=180) were unhatched eggs, used for mycological examination

The potentially pathogenic species, most frequently identified according to the macroscopic and microscopic characteristics were: 13/35 - *Aspergillus niger*, 12/25 - *Aspergillus fumigatus*, 10/35 - *Aspergillus flavus* and 10/18 - *Penicillium crysogenum* and 12/20 - *Fusarium oxysporum*.



Conclusions

The research carried out revealed the presence of an extremely diverse mycotic population in the contents of unhatched eggs.

Among the species isolated, the species of the genus *Aspergillus* (*A. niger*, *A. flavus*, *A. fumigatus*) had the highest frequency.

Other fungal species isolated in this study, which also have pathogenic and toxic potential, belong to the genera *Alternaria*, *Penicillium* and *Fusarium*.

A low egg hatch percentage must also account for primary fungal contamination, which occurs on the farm. In the incubator, fungi find excellent conditions for development that can induce indirect embryo mortality.

It is recommended to use a product, which, applied by fumigation, will also act effectively on fungi, not only on bacteria and viruses.