

Isolation and characterization of mycoflora of chicken hatcheries in Mazandaran province, north of Iran

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Article Info	Abstract
<p>Article history:</p> <p>Received: 28 December 2017 Accepted: 24 July 2018 Available online: 15 December 2018</p> <p>Key words:</p> <p>Contamination Fungus Hatchery Iran</p>	<p>Fungal infections cause significant economic losses in the poultry industry either due to their direct infectious nature or due to mycotoxins production. Hatchery contamination with fungi can threaten chicken health. In this regard, geographical and seasonal distributions of airborne fungal contamination of 25 hatcheries in Mazandaran province, northern Iran, were investigated using an open plate method. The results of this study showed that hatcheries have various fungal contaminations, among which the most common were respectively <i>Cladosporium</i> (31.07%), <i>Penicillium</i> (24.00%), <i>Aspergillus</i> (20.63%), sterile hyphae (14.70%) and <i>Alternaria</i> (6.20%) from different regions. The results revealed that the highest level of fungal isolation was in spring and autumn. This study also showed that the concentration of fungal air spora in forest and seaside locations was significantly greater than mountainous ones. In spite of the regular disinfection in commercial hatcheries, fungal contamination was found in different parts.</p>

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جداسازی و شناسایی فلور قارچی جوجه کشی های استان مازندران در شمال ایران

چکیده

عفونت‌های قارچی به دلیل ماهیت عفونی مستقیم آنها یا به علت تولید میکوتوکسین‌ها، باعث زیان‌های اقتصادی قابل توجه‌ای در صنعت طیور می‌شوند. آلودگی جوجه‌کشی با قارچ‌ها می‌تواند سلامت جوجه را تهدید کند. در این راستا، توزیع‌های جغرافیایی و فصلی آلودگی قارچی هوابرد ۲۵ جوجه‌کشی استان مازندران در شمال ایران با استفاده از روش پلیت باز مورد ارزیابی قرار گرفت. نتایج این مطالعه نشان داد که جوجه‌کشی‌ها دارای آلودگی‌های قارچی متنوعی می‌باشند که در میان آنها شایع‌ترین آلودگی به ترتیب متعلق به کلادوسپوریوم (۳۱/۰۷ درصد)، پنی‌سیلیوم (۲۴/۰۰ درصد)، آسپرژیلوس (۲۰/۶۳ درصد)، هیفای استریل (۱۴/۷۰ درصد) و آلترناریا (۶/۲۰ درصد) از مناطق مختلف بود. نتایج نشان داد که بیشترین میزان جداسازی قارچ در بهار و پاییز بود. این مطالعه همچنین نشان داد که میزان اسپور قارچ در مناطق جنگلی و ساحلی به طور معنی‌داری بیشتر از مناطق کوهستانی بود. با وجود ضد عفونی منظم جوجه‌کشی‌های صنعتی، آلودگی قارچی در بخش‌های مختلف یافت شد.

واژه‌های کلیدی: آلودگی، ایران، جوجه‌کشی، قارچ

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Introduction

Hatchery hygiene is recognized as a critical factor in healthy poultry production. Fungi are among the most common organism in all environments. Zureik *et al.* have reported the airborne fungal concentration to have a profound influence on the respiratory health of both human and animals.¹ Therefore, contamination of poultry hatcheries by micro-organism can adversely affect chick quality and cause embryonic death. The predisposing factors for fungal growth and dissemination in air/environment include humidity, warm environment and poor ventilation, especially for *Aspergillus* species which are highly ubiquitous and a known cause of embryo mortality in hatcheries.²

A large number of fungi (such as *Aspergillus*, *Penicillium* and *Fusarium*) produces mycotoxins and/or secondary metabolites and volatile organic compounds affecting human and animal health. Aspergillosis, commonly known as brooder's pneumonia, is caused mainly by *Aspergillus fumigatus*, the most pathogenic fungi affecting poultry, causes high morbidity and mortality especially in young chicks. Several fungal genera have been shown to cause allergy, such as *Aspergillus*, *Alternaria* and *Cladosporium* and can cause allergic respiratory disease, especially asthma. This study was conducted to determine and summarize both geographical and seasonal distributions of airborne fungus species isolated from hatcheries in Mazandaran province in north of Iran.

Materials and Methods

Research regions. Mazandaran province is surrounded by Alborz Mountain along with the southern coast of the Caspian Sea in the central north of Iran and is mostly rainy and temperate. The study was performed in 25 broiler hatcheries. The examined hatcheries were distributed in seven different cities including Amol (36.4676° N, 52.3507° E), Babolsar (36.7049° N, 52.6547° E), Ghaemshahr (36.4684° N, 52.8634° E), Nowshahr (36.6494° N, 51.4887° E), Tonekabon (36.8155° N, 50.8716° E), Sari (36.5659° N, 53.0586° E) and Juybar (36.6454° N, 52.9010° E) in Mazandaran province in three different geographical areas (mountainous, coastal and forestal). Sampling was conducted during a period of one year and done once per season. Almost equal numbers of hatcheries were considered in each area. Nine hatcheries were located in the coastal areas (three in Nowshahr, two in Sari, two in Tonekabon, one in Juybar and one Babolsar), eight hatcheries were in the mountainous region (three in Sari, three in Amol and two in Tonekabon) and eight were in the forestal areas (three in Amol, two in Sari, two in Tonekabon, and one in Ghaemshahr).

Sampling and isolation methods. Sampling was carried out using sterile plastic Petri dishes containing Sabouraud's dextrose agar (Oxoid CM0041; Oxoid Ltd., Basingstoke, UK) and chloramphenicol (0.005 mg) placing uncovered for 10 min at a height of one meter from the floor surface in setter and hatchery rooms.³

Totally, 3639 fungal colonies were isolated on plates. The samples were maintained at incubator at 30 °C for 7-14 days. The morphological characters of the fungal isolates were studied by the direct method using lacto phenol cotton blue for preliminary identification.⁴

Statistical analysis. Data were analyzed using SPSS (version 16.0; SPSS Inc., Chicago, USA). Descriptive analysis was calculated. Paired *t*-test was used to investigate the trend of fungal changes in setter and hatchery and evaluate the association among cities, capacity of hatcheries, different geographical locations and seasons. The one-way ANOVA analysis was used as well. Factors with *p*-value less than 0.05 were analyzed using post hoc test (Bonferroni). In all analyses, *p* < 0.05 was considered as statistically significant.

Results

Table 1 presents the variety of flora accruing in different hatcheries. The predominant fungi isolated from setter and hatchery of incubators belonged to genus *Cladosporium* (31.07%), followed by *Penicillium* (24.00%), *Aspegillus* spp. (20.63%) which 6.00% and 8.40% of them were *Aspergillus flavus* and *Aspergillus fumigatus*, respectively, sterile hyphae (14.70%) and *Alternaria* (6.20%). As summarized in Table 1, *Penicillium* and *Cladosporium* (25.08% and 18.90%), (14.40% and 40.62%) and (37.76% and 19.71%) in mountainous, coastal and forestal areas were the most predominant fungi, respectively. The abundance of *Cladosporium* spp. in coastal areas was greater than two other areas. This association was statistically significant between coastal and mountainous areas (*p* < 0.05).

Although the concentration of air spora in the forestal and coastal locations was significantly greater than mountainous one (*p* < 0.05), *Cladosporium* spp. and *Penicillium* spp. had a high prevalence in all three geographical areas. Presence of some important pathogenic fungi such as *Aspergillus flavus*, *Aspergillus fumigatus* and *Mucor* was noticeable. In particular, *A. flavus* and *A. fumigatus* had a high frequency in coastal and forestal areas and the difference was statistically significant in both coastal and mountainous regions (*p* = 0.05).

The Presence of fungi in different seasons in Table 2 shows that the most isolated fungi in hatcheries are *Penicillium* and *Cladosporium* (34.43% and 30.37%) in spring, *Rodotorella* and *A. clavatus* (44.72% and 24.96%) in summer, *Cladosporium* and sterile hyphae (33.83% and 23.35%) in autumn and *Penicillium* (43.85%) in winter.

Table 1. Frequency of fungal colonies in three geographical regions.

Organism	Mountainous	Coastal	Forestal
	No. plates positive (%)	No. plates positive (%)	No. plates positive (%)
<i>Aspergillus flavus</i>	21 (7.21)	145 (6.74)	99 (6.61)
<i>Aspergillus fumigatus</i>	25 (8.59)	203 (9.44)	101 (6.75)
<i>Aspergillus niger</i>	2 (0.68)	20 (0.93)	7 (0.46)
<i>Aspergillus clavatus</i>	0 (0)	0 (0)	1 (0.06)
<i>Aspergillus candidus</i>	4 (1.37)	1 (0.04)	4 (0.26)
<i>Aspergillus sydowii</i>	0 (0)	0 (0)	2 (0.13)
<i>Aspergillus versicolor</i>	0 (0)	7 (0.32)	0 (0)
<i>Aspergillus terreus</i>	0 (0)	0 (0)	2 (0.13)
<i>Penicillium spp.</i>	73 (25.08)	304 (14.14)	565 (37.76)
<i>Fusarium spp.</i>	5 (1.71)	14 (0.65)	14 (0.93)
<i>Cladosporium</i>	55 (18.90)	873 (40.62)	295 (19.71)
<i>Rodotorolla</i>	4 (1.37)	15 (0.69)	28 (1.87)
Sterile hyphae	40 (13.74)	272 (12.65)	266 (17.78)
<i>Monoascus ruber</i>	3 (1.03)	51 (2.37)	13 (0.86)
<i>Altrenaria spp.</i>	23 (7.90)	172 (8)	49 (3.27)
<i>Scopulariopsis</i>	1 (0.34)	2 (0.09)	2 (0.13)
<i>Mocur spp.</i>	2 (0.68)	8 (0.37)	3 (0.20)
<i>Ulocladium spp.</i>	1 (0.34)	4 (0.18)	2 (0.13)
<i>Rhizopus spp.</i>	1 (0.34)	6 (0.27)	1 (0.06)
<i>Stemphylium spp.</i>	0 (0)	0 (0)	2 (0.13)
<i>Aureobasidium spp.</i>	0 (0)	0 (0)	1 (0.06)
<i>Candidia spp.</i>	20 (6.87)	0 (0)	2 (0.13)
<i>Scytalidium spp.</i>	0 (0)	0 (0)	2 (0.13)
<i>Geomyces spp.</i>	2 (0.68)	0 (0)	0 (0)
<i>Pseudallescheria spp.</i>	2 (0.68)	0 (0)	0 (0)
<i>Madurella spp.</i>	0 (0)	2 (0.09)	0 (0)
<i>Sporotrichum spp.</i>	0 (0)	7 (0.32)	1 (0.06)
<i>Dactylaria spp.</i>	0 (0)	1 (0.04)	0 (0)
<i>Epicoccum spp.</i>	0 (0)	1 (0.04)	0 (0)
<i>Syncephalastrum spp.</i>	0 (0)	4 (0.18)	0 (0)
<i>Trichothecium spp.</i>	0 (0)	0 (0)	1 (0.06)
<i>Absidia spp.</i>	7 (2.40)	31 (1.44)	24 (1.60)
Total	291 (100)	2149 (100)	1496 (100)

However, varieties of fungi were higher in spring than other seasons. Sampling from different hatcheries revealed significant seasonal difference in *Cladosporium* spp. ($p < 0.05$). The *A. flavus* and *A. fumigatus* were observed in all seasons. However, their frequency in the autumn was significantly higher than other seasons ($p < 0.05$). *Cladosporium* and *Penicillium* in high capacity (49.36% and 17.87%), in medium capacity (23.20% and 25.44%) and in low capacity (19.30% and 26.30%) hatcheries were the most isolated fungi. There was no statistically significant difference between different capacity hatcheries.

The *A. flavus* and *A. fumigatus* were observed in all three capacity hatcheries. However, their prevalence in medium capacity was much higher than low and high capacity hatcheries.

Aspergillus fumigatus (34.14%) and *Aspergillus falvus* (22.10%) in Nowshahr hatcheries, *Penicillium* (66.48%) and *Aspergillus fumigatus* (21.42%) in Qaemshahr, *Penicillium* (31.32%) in Amol and *Cladosporium* in Tonekabon (68.35), Juybar (63.26%) and Babolsar

(34.23%) were predominant isolated fungi. A significant statistical difference between the prevalence of *Cladosporium* and *Aspergillus fumigatus* in different cities was observed ($p < 0.05$).

Discussion

Environmental contamination of hatcheries with fungal spores may be due to contamination of hens and thus the production of contaminated eggs. In addition, the temperature and humidity of egg incubator facilitate penetration of egg shell by spores. In the last stages of embryonic development, the egg shell porosities increase, especially at the blunt end of the egg. Hence, it raises the chance of spores entering to the egg. This explains observation of fungal growth at the blunt end.⁵

There are a large number of laying, broiler breeding, broiler and hatchery farms in Mazandaran province in north of Iran because of its favorable climatic condition. The humidity is high and climate temperature favors fungal growth.

Table 2. Frequency of fungal colonies in different seasons.

Organism	Spring	Summer	Autumn	Winter
	No. plates positive (%)	No. plates positive (%)	No. plates positive (%)	No. plates positive (%)
<i>Aspergillus flavus</i>	19 (1.54)	11 (1.36)	192 (14.30)	43 (10.77)
<i>Aspergillus fumigatus</i>	49 (3.99)	39 (4.84)	233 (17.36)	8 (2)
<i>Aspergillus niger</i>	7 (0.57)	5 (0.62)	16 (1.19)	1 (0.25)
<i>Aspergillus clavatus</i>	1 (0.08)	201 (24.96)	0 (0)	0 (0)
<i>Aspergillus candidus</i>	4 (0.32)	0 (0)	5 (0.37)	0 (0)
<i>Aspergillus sydowii</i>	0 (0)	0 (0)	2 (0.14)	0 (0)
<i>Aspergillus versicolor</i>	0 (0)	0 (0)	7 (0.52)	0 (0)
<i>Aspergillus terreus</i>	2 (0.16)	0 (0)	0 (0)	0 (0)
<i>Penicillium spp.</i>	386 (31.43)	45 (5.59)	18 (1.34)	175 (43.85)
<i>Fusarium spp.</i>	15 (1.22)	1 (0.12)	10 (0.74)	4 (1)
<i>Cladosporium</i>	373 (30.37)	9 (1.11)	454 (33.83)	36 (9.02)
<i>Rodotorolla</i>	37 (3.01)	360 (44.72)	6 (0.44)	3 (0.75)
Sterile hyphae	108 (8.79)	4 (0.49)	313 (23.32)	112 (28.07)
<i>Monoascus ruber</i>	58 (4.72)	120 (14.90)	0 (0)	0 (0)
<i>Altrenaria spp.</i>	46 (3.74)	0 (0)	66 (4.91)	12 (3)
<i>Scopulariopsis</i>	3 (0.24)	2 (0.24)	2 (0.14)	0 (0)
<i>Mocur spp.</i>	8 (0.65)	0 (0)	2 (0.14)	0 (0)
<i>Ulocladium spp.</i>	6 (0.48)	1 (0.12)	1 (0.07)	0 (0)
<i>Rhizopus spp.</i>	4 (0.32)	0 (0)	2 (0.14)	1 (0.25)
<i>Stemphylium spp.</i>	2 (0.16)	0 (0)	0 (0)	0 (0)
<i>Aureobasidium spp.</i>	1 (0.08)	0 (0)	0 (0)	0 (0)
<i>Candidia spp.</i>	22 (1.79)	0 (0)	0 (0)	0 (0)
<i>Scytilidium spp.</i>	2 (0.16)	0 (0)	0 (0)	0 (0)
<i>Geomyces spp.</i>	2 (0.16)	0 (0)	0 (0)	0 (0)
<i>Pseudallescheria spp.</i>	2 (0.16)	0 (0)	0 (0)	0 (0)
<i>Madurella spp.</i>	2 (0.16)	0 (0)	0 (0)	0 (0)
<i>Sporotrichum spp.</i>	7 (0.57)	0 (0)	1 (0.07)	0 (0)
<i>Dactylaria spp.</i>	1 (0.08)	0 (0)	0 (0)	0 (0)
<i>Epicoccum spp.</i>	1 (0.08)	0 (0)	0 (0)	0 (0)
<i>Syncephalastrum spp.</i>	1 (0.08)	3 (0.37)	0 (0)	0 (0)
<i>Trichothecium spp.</i>	0 (0)	0 (0)	1 (0.07)	0 (0)
<i>Absidia spp.</i>	56 (4.56)	3 (0.37)	4 (0.29)	0 (0)
Total	1228 (100)	805 (100)	1342 (100)	399 (100)

Different types and species of fungi were isolated from hatcheries. Thirty-three species representing twenty-five genera were identified (Table 1). *Cladosporium*, *Penicillium* and *Aspergillus* were the predominant flora in hatcheries, similar results were found in studies investigating fungal flora in different places.⁶⁻⁸ Study of Ajoudanifar *et al.* at poultry and cattle houses in Mazandaran have revealed that *Cladosporium* (55.30%), Yeast (10.00%) and *Aspergillus* (9.40%) were the most common findings, respectively.⁹

In the winter, a minimum level of fungi was found due to low temperature and little outdoor vegetation which would have influenced fungal growth. Similarly, the study of Sen and Asan on the seasonal distribution of fungal flora in Tekirdag city, Turkey, has showed that maximum fungi are isolated in spring and autumn.¹⁰ In their study, temperature, humidity and vegetation also showed their highest levels during these periods, which means a very suitable condition for fungal growth. There have been many reports concerning the seasonal variation of fungi in different countries. Most studies have indicated that the

peaks of fungi concentration are recorded during summer and early fall months.¹¹ Similar results were reported by Soliman *et al.* in closed housing during summer.¹² Several studies have indicated seasonal occurrence of fungal disease (Aspergillosis) in waterfowls with higher incidence in spring and autumn.¹³

Although the diversity of fungi in the forestal areas was higher than the other two ones, the variety of fungi in different regions was not statistically significant. Fungal colonies in coastal and forestal areas were higher than mountainous area. Result of many studies has showed that 60.00 to 70.00% relative humidity and temperature between 15 to 25 °C in outdoor air increase the number of fungal spores. Regarding temperature and humidity, forestal and coastal areas conditions are more suitable for fungal growth.¹⁴

The medium capacity hatcheries were higher in colony count, fungal diversity and pathogen fungi than high and low hatcheries. The results showed that the capacity of hatcheries cannot affect fungal flora may be due to hygienic measures and management practice.

The most prevalent species across all hatcheries were *Cladosporium* spp. It is known that *Cladosporium* is the most widespread airborne fungus of the mild climate zone. Burch and Levetin have reported that *Cladosporium* was high in several studies.¹⁵ Our finding was in agreement with results of Eckman and Jones.¹⁶ They have investigated the fungal flora of hatchery. Its potential pathogenicity in chickens is unknown, however, it has been reported that *C. herbarum* produces dermatitis in chickens. Total number of fungi, especially pathogenic fungi was higher in the coastal areas perhaps due to better conditions for fungi growth regarding humidity and temperature.

Mainly identified pathogenic fungi were *Aspergillus flavus* and *Aspergillus fumigatus*, while others like *Candidia*, *Dactylaria* and *Fusarium* were isolated to a lesser extent. It is well known that *Aspergillus*, *Penicillium* and *Fusarium* are the genera of fungi which most implicated from mycosis or mycotoxins. Mycotoxins produced by *Aspergillus*, *Penicillium* and *Fusarium* have been found toxic and lethal for chick embryos.¹⁷

Penicillium reached its maximum colonies in spring (31.43%) and in forestal area (37.76%). It produces one or more mycotoxins including ochratoxin which is hepatotoxic in domestic animals.¹⁸ Previous study has also revealed the association between *Penicillium oxalicum* and embryo mortality.⁵

In the present study, most colonies of *Aspergillus flavus* and *fumigatus* were found in autumn and in coastal areas. *Aspergillus* grows in lower humidity and high temperatures; therefore it reaches its maximum spore levels in the summer, which is in conflict with our results.¹⁰

Aspergillosis has been described in mammals, human, domestic animals and wild birds. It is commonly known that *Aspergillus fumigatus* causing brooder's pneumonia is the most pathogenic fungi affecting poultry.

In 95.00% of the Aspergillosis cases in wild birds of prey, *Aspergillus fumigatus* was detected. As *A. fumigatus* was the predominant fungus isolated from dead eggs and fecal material, it was appeared to be responsible for hatchability reduction.⁵

Source of *Aspergillus* in hatcheries could be utilities, poultry workers, contaminated eggs and air flow. *Aspergillus fumigatus* has the smallest spores among *Aspergillus* spp. Therefore, compared to others, its infection occurs more frequently in poultry. In this study, the predominance of *Aspergillus* species, especially *A. flavus* and *A. fumigatus* in two cities, (i.e, Nowshahr and Amol) were observed.

The presence of fungi in hatcheries is also important from the point of view of pathogenicity for hatcheries workers. The human exposure to airborne dust and microorganism such as bacteria and fungi can cause respiratory disease.¹⁹ According to the European Community of Respiratory Health Survey results; fungal allergy is a strong risk factor for severe asthma in work

places.¹ *Cladosporium herbarum* is one of the most common species to be isolated. Association of sensitization, exposure to indoor airborne *Penicillium* through wheeze and severity of asthma have been confirmed by former studies.²⁰

In conclusion, as microorganism in the hatchery has a serious impact on chicks' viability and quality as well as overall growth performance, hygienic measures in the management of a hatchery for increased profitability in the poultry industry are significant and need no emphasis.

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Conflict of interest

The authors do not have any potential conflicts of interest to declare.

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