

## 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

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### Abstract

This study is conducted at the Veterinary Directorate in Duhok Province for (12) months and it has been done on its Veterinary Laboratory in Duhok Governorate, Kurdistan Region of Iraq.

The main aspect of the research is to isolate *Aspergillus fungi*, other fungus and bacteria (*Salmonella* and *E. coli*) in the veterinary laboratory In order to know what kinds of fungi and bacteria are occur in Poultry farm and Hatchery in Duhok area.

It divided fungi into three groups, one group includes *Aspergillus*, second group is other fungus, and the third group is to isolate *Salmonella* and *E. coli* Bacteria.

Researchers thought the poultry farms and hatcheries will not occur fungi, but after diagnosis it appears a huge quantity of fungus, especially *Aspergillus* and it affect slightly on poultry industry sector in Duhok area. But, fortunately, it does not occur bacteria, which has not impact negatively of poultry industry sector in Duhok area.

The majority of samples were resulted negative in *Aspergillus* and other fungus; however, there were no bacteria occur in any samples in both poultry farms and poultry hatcheries.

This indicates that the poultry farms and hatcheries are contaminated from fungus but not bacteria.

In our study there weren't significantly differences in species of fungus, and it has no obvious explanation because of difference in species of fungus. It may get curious result that needs further investigation. Perhaps this reflects types, level and source of fungi, birds, the way of using disinfection, and variety to name but a few of the variables that could be investigated. The study also led us to follow up the research, recording more data and continuing research in the future.

**Keywords:** *Aspergillus*; other fungi; *Salmonella*; *E. coli* Bacteria; Poultry farms and Poultry Hatcheries

### Introduction

Fungal/mycotic diseases cause significant economic losses to the poultry industry sector either due to their direct infectious nature or due to production of mycotoxins, the secondary fungal metabolites produced in grains or poultry feed. Several fungi have created havoc in the poultry industry and some of them cause direct harm to human health due to their zoonotic implications [1]. They are responsible for high morbidity and mortality, especially in young birds and cause stunted growth and diarrhea; and fatal encephalitis. Mycotic dermatitis is a possible health hazard associated with poultry farms [1].

Mycotoxins are the leading cause of producing immunosuppression in birds, which makes them prone to several bacterial and viral infections leading to huge economic losses to the poultry industry sector. In comparison to bacterial and viral diseases, advances in diagnosis, treatment, prevention and control of fungal diseases in poultry have not taken much attention. Recently, molecular biological tools have been explored for rapid and accurate diagnosis of important fungal infections [2]. Effective prevention and control measures include: appropriate hygiene, sanitation and disinfection, strict biosecurity program and regular surveillance/monitoring of fungal infections as well as following judicious use of anti-fungal drugs. Precautionary measures during crop production, harvesting and storing and in feed mixing plants can help to check the fungal infections including health hazards of mycotoxins/mycotoxicosis [2].

The present review describes the fungal pathogens causing diseases in poultry/birds, especially focusing to their diagnosis, prevention and control measures, which would help in formulating appropriate strategies to have a check and control on these unwanted troubles to the poultry producers/farmers [2].

Aspergillosis is a fungal infection of the respiratory tract in young birds, also commonly known as 'brooder pneumonia'. In infected birds, the air sacs and lungs show white to yellow spots or lesions. Infected birds gasp for air and have accelerated breathing. Aspergillosis can also result in severe eye (and even brain) infection, which can appear as a yellow cheesy pellet beneath the eyelid. Increased mortality rates of 5 to 50 % may occur within 21 days after the placement of diseased birds [2].

Day-old chicks with signs of Aspergillosis are infected by the spores of *Aspergillus* species, among which *Aspergillus fumigatus* is the most common. The spores of the fungus *Aspergillus* are like small, dry seeds that can easily be spread by draught or the wind. The spores are found in low numbers throughout the whole environment. *Aspergillus* spores survive and grow in a wide range of conditions, but especially on organic matter, like egg yolk, cardboard boxes and wood. Growth in the spores is initiated by conditions of high humidity and temperature (37 - 45°C). Cycles of high and low humidity optimize the growth of the fungus (mycelium) and the spread of its spores. The hatchery therefore provides optimum environmental conditions for *Aspergillus* to thrive.

*Aspergillus* spores can enter the hatchery either directly via the eggs, or via incoming air. When the hatchery environment is contaminated by a high level of *Aspergillus*, the spores can easily enter the air handling unit and ventilation system. The climate, temperature and humidity in ventilation ducts are ideal for the propagation of *Aspergillus*, especially when organic matter (debris) has accumulated.

The main route into the hatchery for *Aspergillus* spores however, is usually via contaminated eggs. *Aspergillus* spores attached to the shell find their way into the albumen and yolk via hairlines or cracks in the shell. The yolk of the egg is an ideal substrate for the growth of *Aspergillus* and once the spores have entered the eggs, the way to the hatchery is open.

The nests also contain several sources of *Aspergillus*, including bedding, manure and feed. The nest eggs therefore become contaminated by contact with *Aspergillus* spores from these sources. Floor eggs also, of course, have a high risk of being heavily contaminated, when the spores enter the egg via hairline and other cracks.

Initially, infection by *Aspergillus* will be found during the routine analysis of unhatched eggs. Infected eggs show a visible mould on the membrane in the air cell (see picture). The risk of a serious bloom of *Aspergillus* is high when 0.5 % of the hatching eggs show clear infection with a visible growth of the fungus. Also, the frequency of embryos dying at about day 16 is higher than expected.

Other fungi are also effect negatively on poultry shed and hatchery that causes economically impacted on income of the owner's poultry farm [3,4].

A hyper-producing strain of *Aspergillus niger* var teigham was used for the production of phytase in shake flask and fermenter [5].

The *Salmonella* and *E. coli* are also affected negatively on poultry farms and hatcheries in many areas especially in Duhok city in Kurdistan Region of Iraq [3]. *Salmonella's* bacteria is other than the species specific sero-types *S. pullorum*, *S. gallinarum*, and also other than *S. enteritidis* and *S. typhimurium* (which are considered separately), are capable of causing enteritis and septicaemia in young birds. Serotypes vary, but S. Derby, S. Newport, S. Montevideo, S. Anatum, S. Bredeney are among the more common isolates. Even if these infections do not cause clinical disease, their presence may be significant with respect to carcass contamination as a potential source of human food poisoning. They infect chickens, turkeys and ducks worldwide [6,7].

*E. coli* enteritis is swelling (inflammation) of the small intestine from *Escherichia coli* (*E. coli*) bacteria. It is the most common cause of travelers' diarrhea [2].

Because of the wide variety of serotypes involved and the diverse sources of infection, strategies are necessary to prevent the risk of contaminating flocks. As the eradication of Salmonellas in poultry is an utopia, some serotypes need to be eliminated due to their economic damage (S.g and S.p) or their impact on public health (*S. enteritidis*, *S. typhimurium*, *S. heidelberg*). As these specific serotypes are strongly related to the reproductive tract of female birds it is necessary to rigorously break the cycle within these hens. Serotypes that do not use the transovarial route usually remain in the intestine and their eradication requires strict control measures focusing on environment factors (rodents, flies, feed, pets, etc.) [7].

## Materials and Methods

The study is conducted at Veterinary Directorate in Duhok Governorate. It used their device and materials that belongs to the General Directorate of Animal Resources and Veterinary – the Ministry of Agriculture and Water Resources in Kurdistan Region of Iraq.

It was carried out to ascertain the incidence of *Aspergillus fumigatus* in commercial poultry hatchery during different seasons. The sampling period was from January to December 2015 for investigation of the prevalence of *Aspergillus fumigatus* in commercial poultry hatchery house.

These samples were directly streaked on sabouraud agar plates for culturing and were incubated for 7 days at 37°C. *Aspergillus fumigatus* was identified according to its specific colony characteristics, slides were also prepared for identification of mycelium and hyphal arrangement with lactophenol blue staining method (Darise, 1987).

Darise. H. L. (1987) medically important Fungi. A guide to identification. P. 14-15.

They have been chosen three main groups, two groups of fungus (one group of *Aspergillus*'s fungi and the second group of other fungi), the third group is isolated *Salmonella* and *E. coli* bacteria in poultry farms and poultry hatcheries as well.

Certain teams from different district were taken a sample from farms and hatcheries then transfer into Veterinary laboratory in order to diagnose from fungus and bacteria.

## Isolation *Salmonella* and *E. coli*

Six hundred and sixty three samples were collected from different hatcheries and farms in Duhok Governorate.

The samples were carried to the laboratory in clean container, and then the samples were suspended in 10 ml nutrient broth and incubate for 24 hrs at 37°C for pre-enrichment. 1 ml of pre-enrichment broth was transfer into 10 ml of Rappaport broth (CONDA, Spain) and incubated for 24 hrs at 42°C. One loop-full of Rappaport broth was streaked onto *Salmonella Shigella* (55) agars (CONDA, Spain) and brilliant green agar (CONDA, Spain), YLD agar (CONDA, Spain) and incubated at 37°C for 24 hrs. The suspended colonies were transferred to ABC chromogenic agar (CONDA, Spain) and incubated for 24 hrs at 37°C.

As same salmonella loop – full of pre-enrichment broth (nutrient broth) were streaked onto MacConkey agar (CONDA, Spain), EMB agar (CONDA, Spain) and incubated for 24 hrs at 37°C.

**Results**

Throughout the period (10 days), it indicated that (table1) the number of fungus increased slightly in both poultry farms and poultry hatcheries even the differences in species of fungi in each area of the city with similar number of fungus.

However, the *Salmonella* and *E. coli* bacteria have not occurred in any sample, and it shows the negative result in both poultry farms and hatcheries.

Table 1 and table 2 (The positive and negative result of fungus and bacteria in both poultry farms and poultry hatcheries in the Vet. lab.)

#	<i>Aspergillus fungi</i>					
	Name of Shed OR Hatchery	No. of Sample	+Ve	-Ve	Slides	Other Fungi
1	Solav Hatchery	126	20	106	54	34
2	Zheeen Hatchery	162	18	144	57	48
3	Bade Hatchery	73	17	56	43	30
4	Mokhtar Hatchery	178	28	150	53	47
5	Khilikh Shed	3	3	0	3	1
6	Bisheesh Shed	6	2	4	2	0
7	Botia Shed	8	6	2	6	0
8	Baadalia Hatchery	59	13	43	47	42
9	Garza Shed	5	4	1	4	1
10	Dilshad Shed	2	1	1	1	0
11	Piromara Shed	4	0	4	0	0
12	Dost Shed	1	1	0	1	0
13	Aloka Shed	1	0	1	0	0
14	Darvan Shed	4	4	0	4	0
15	Doban Shed	2	0	2	0	0
16	Bablo Shed	1	0	1	0	0
17	Waleed Shed	4	0	4	0	0
18	Hysean Shed	5	0	5	0	0
19	Sarky Shed	2	0	2	0	0
20	Jagerkhwen1 Shed	5	0	5	0	0
21	Marena Shed	1	0	1	0	1
22	Bnave Shed	5	0	5	1	2
23	Jagerkhwen2 Shed	5	0	5	1	1
24	Laween Shed	1	0	1	0	0
	Total	663	118	545	277	234

**Table 1:** The Diagnose result of *Aspergillus* Fungi and other fungus at Vet. Laboratory in Duhok, Kurdistan Region.

#	Name of Shed OR Hatchery	No. of Sample	Isolation of <i>Salmonella</i> and <i>E. coli</i>
1	Solav Hatchery	126	-Ve
2	Zheeen Hatchery	162	-Ve
3	Bade Hatchery	73	-Ve
4	Mokhtar Hatchery	178	-Ve
5	Khilikh Shed	3	-Ve
6	Bisheesh Shed	6	-Ve
7	Botia Shed	8	-Ve
8	Baadalia Hatchery	59	-Ve
9	Garza Shed	5	-Ve
10	Dilshad Shed	2	-Ve
11	Piomara Shed	4	-Ve
12	Dost Shed	1	-Ve
13	Aloka Shed	1	-Ve
14	Darvan Shed	4	-Ve
15	Doban Shed	2	-Ve
16	Bablo Shed	1	-Ve
17	Waleed Shed	4	-Ve
18	Hysean Shed	5	-Ve
19	Sarky Shed	2	-Ve
20	Jagerkhwen1 Shed	5	-Ve
21	Marena Shed	1	-Ve
22	Bnave Shed	5	-Ve
23	Jagerkhwen2 Shed	5	-Ve
24	Laween Shed	1	-Ve
	Total	663	0

**Table 2:** The Diagnose result of *Salmonella* and *E.coli* Bacteria at Vet. Laboratory in Duhok, Kurdistan Region.

## Discussion

In our study there weren't significantly differences in occur and exist *Aspergillus*, other fungus and *salmonella* and *E. coli* bacteria in both poultry farms and poultry hatcheries in different districts around the Duhok city. The majority of samples were resulted positive in *Aspergillus* and other fungi; however, there were negative result of bacteria occur in any samples in both poultry farms and poultry hatcheries.

It has no obvious explanation because of huge number of poultry farms and certain of poultry hatcheries in Duhok government [3]. It is a curious result that needs further investigation. As shown in previous study (*S. enteritidis*, *S. typhimurium*, *S. Heidelberg* and Mustafa SO. 2013 [3]). Further study required to know the exact district in order to isolated the bacteria species that seen the may effected on poultry farms and hatcheries owner economic [8-15].

## Conclusion and Recommendation

From the results, it is an obvious that the *Aspergillus* and other fungus are exist but *Salmonella* and *E. coli* bacteria are not occur in both poultry farms and poultry hatcheries in different districts around Duhok city that have a different impact on each group of fungi and bacteria in terms of different district.

In contrast, study should include the species of fungi and specific district in order to know which kind of fungus have significantly impact on decreasing the income of the farms and hatcheries owners.

Overall it can be concluded that the addition of data that contain majority areas in duhok city can potentially improve owners economic, reduce the cost of treatment. On the other hand, for this to be achieved additional research to look at the efficiency of this collect data on fungi and bacteria of complete lab. Based on exact species of fungi and bacteria is required.

## Future Research

They are a number of areas that require further research and some have been outlined above. The important consideration must be to match the species of fungi and bacteria in the combination profile of the poultry farms and poultry hatcheries. Why do fungi and bacteria not give positive responses in all situations? Perhaps this reflects type, level and source of fungi, bacteria variety to name but a few of the variables that could be investigated.

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