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ANALYSIS OF ENVIRONMENTAL CONTAMINATION WITH BACTERIA AND FUNGI IN A BROILER HOUSE IN QENA, EGYPT

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ABSTRACT

Environmental quality is much correlated with animal welfare. Microbiological status of a chicken broiler house environment in Qena city, Upper Egypt was investigated for total aerobic bacteria, fungal count, total coliforms, Escherichia coli, fecal enterococci, Staphylococci and Pseudomonads. Bacterial and fungal cultures from poultry feed, water, Litter, and air were undertaken every week throughout the 7-wk fattening period. The results showed that the indoor microbial contamination were generated and detected. It was revealed that microorganisms in air were detected at a mean value (log_{10} cfu/m^3) of 4.17 for total aerobic bacteria; 1.86 for total coliforms; 0.89 for E. coli; 1.59 for fecal enterococci; 2.78 for Staphylococci; 2.16 for Pseudomonads; and 2.59 for yeast/mold. The predominant bacterial species include S. aureus, Pseudomonas aeruginosa; fecal enterococcus, E. coli meanwhile Aspergillus flavus, Aspergillus fumigatus, and Candida albicans were among the mostly encountered fungal species. The results indicated an increase in air microbial concentration correlated with growth and age of birds. Microbiological examination of samples from litter, feed and water revealed contamination with one or more of microbial groups or species investigated. The present study revealed the occurrence of microbial contamination in poultry environment and strict hygienic measures should be considered for bird health performance and human and surrounding environment as well.

Keywords: broiler house; contamination; environmental; bacteria; fungi

INTRODUCTION

Nowadays, one of the most important contaminants in the farming environment are bioaerosols. In poultry intensive production, chicken fattening in particular, is a significant source of air and surfaces contamination whereas many of birds housed and the haze of suspended dirt, feces, feathers and skin fragments, that may adversely affect the animal health as well as may pose a health risk for animal caretaker and those living in close proximity ***(Donham, 1993)***.

Environmental air monitoring programs can be employed to reduce unsanitary conditions in animal houses due to suspended bacterial and fungal particles in the air in order to assure a good environmental quality. Air quality with regard to poultry production, bird health and environmental contamination has been a major concern for years ***(Ritz et al., 2006)***. Poultry are known to harbor pathogenic microorganisms, and as such poultry house air can be contaminated with these microorganisms. A lot of microorganisms including bacteria, viruses, fungi, and parasites that can spread via air among animals and from animals to humans.

Health concerns impact not only the health of the birds, but also can affect the health of humans if they are exposed to high concentrations of bacteria and fungi as well as endotoxins and mycotoxins produced by them. There is epidemiological evidence that the health of farmers working in animal houses may be harmed by regular exposure to the highest concentrations of air contaminants such as gases, dust, microorganisms and endotoxins ***(Whyte et al., 1993)***.

Airborne microbial populations and aerosol production has been examined in broiler houses, hatcheries and egg processing facilities ***(Clark et al., 1983; Sotohy, 1989; Whyte et al., 2001; Northcutt et al., 2004; Karwowska, 2005; Byomi and Trabees, 2006; Duan et al., 2008).***

For these reasons, it is important to analyze the microbiological status of poultry house environment for contamination.

The present work aimed to describe the relative abundance of bacteria, and fungi in a broiler poultry house. A 7 week surveillance study was undertaken for the detection on fungal and bacterial selected target species in the air, food, and litter. Water samples from drinker were evaluated for microbial quality. Selected target bacterial species including coliforms, *E. coli*, fecal enterococci, Staphylococci and Pseudomonads were assessed. Thermophilic fungal species including *Aspergillus* spp. and *C. albicans*, that are potentially pathogenic for birds were assessed.

MATERIALS AND METHODS

Collection of samples:

Measurements were conducted in a chicken broiler house, Qena province, Egypt. The house was visited weekly throughout the fattening period. The house characterized by floor that was fully available to the birds (floor-raised houses), naturally ventilated through windows along the sides and by fans along the barns. Litter consisted of wood shaving spread in the floor.

Over the 7-wk period of fattening, the environmental contamination by bacterial and fungal species were determined inside the house. Samples from air, litter and poultry feeds as well as water samples from drinker were randomly taken.

Air samples:

Air samples of 60 L/min were collected by impaction method (Spin Air, IUL). The air sampler was placed 1.5 m from the ground level.

Litter and feed samples:

One gram of each litter or feed sample was collected and introduced in McCartney tube containing 10 mL of buffered peptone water. After vigorous shaking, 1 ml was used as inoculum.

Water samples:

Water samples were collected from the drinkers under aseptic conditions, then were evaluated for their microbiological quality.

Microbiological Analyses:

The agar plates were prepared and the following media were selected for recovery of total bacterial count Trypticase Soy Agar (TSA) supplemented with cyclohexamide (0.5% Sigma, USA), Staphylococci on Mannitol Salt Agar (MSA), coli-group bacteria on chromocult agar, fecal enterococci on Packers (enterococcus) agar and pseudomonads on cetrimide agar. Fungi were grown on Sabouraud-chloramphenicol dextrose agar plates. All media were from Biolab, Hungary except Chromocoult agar from Merck, Germany.

After sampling, colonies were counted after 48h of incubation at 35 - 37°C for bacteria and after 6 days in 28°C for fungi. The incubation temperature of 40°C was selected to isolates only thermophilic fungal species that may behave as opportunistic pathogens for birds.

The bacterial and fungal contamination were estimated by counting the number of colony-forming units (CFU/ m3 for air samples, g for litter and feed samples). The average number was calculated and transformed into $\log_{10}$ colony forming units.

Water quality evaluation was determined by the most probable number (MPN) of coliforms, *E. coli* and fecal enterococci according to ***(APHA, 1995).***

Isolates identification:

The bacterial isolates were characterized and identified using the methods of ***Cowan and Steel(1990)and Holt et al.(1998)*** and microtests API systems (bioMerieux, Marcy I,Etoiles, France) were also used.

The fungi isolates were identified with the features described by Barnett and Hunter ***(1972; de Hoog et al., 2000).***

Data analysis:

The average concentrations were calculated for each analyzing microbial group or species from duplicate plate counts. All counts were expressed as colony-forming units (CFU/ m3 for air samples, g for litter and feed samples) and transformed into $\log_{10}$ colony forming units since the raw data was not normally distributed. The mean Log_{10} values were determined.

RESULTS

A total of 96 environmental samples were collected as 21 samples from air, 24 from feed, 33 from litter and 18 from water. Bacterial colonies were detected in 14 air samples, 21 litter samples, and 11 feed samples. Fungal colonies were detected in 18 air samples, 21 feed samples, and 29 litter samples.

In general the analyzed air pollutants reached relatively high levels in the mid-fattening period and the airborne microbial contaminants levels at the end of fattening period were almost as those measured at the

beginning (Figure 1). The results of microbiological analyses of air inside broiler house during the 7 week studying period are shown in Table 1. It has been stated that airborne bacteria were recorded at a mean value (log_{10} cfu/m^3) of 4.17 for aerobic bacteria; 1.86 for total coliforms; 0.89 for *E. coli*, 1.59 for fecal enterococci; 2.78 for Staphylococci; and 2.16 for Pseudomonads.

The concentration of airborne fungi was recorded at a mean value of 2.59 log_{10} cfu/m^3.

The microbiological evaluation of feed and litter samples (Table 2 and 3), the results detected a relatively high microbial contamination during the surveillance period.

The result of MPN for water samples from drinkers revealed massive contamination with coliform and fecal enterococci (Table 4).

The bacteriological examination indicated that *Staphylococcus* spp., fecal enterococci, and pseudomonads were the predominant bacterial contaminants recovered from environmental samples; whereas coliforms were in low level and *E. coli* was the most recovered among coliform bacteria (Table 5).

The results of mycological examination of the air , litter and feed samples is recorded in table 6, Aspergillus, Penicillium, Cladosporium and yeasts were the most prevalent genera. The most frequently encountered species were *Aspergillus flavus, A. fumigatus*, and *Candida albicans*. Other fungi were also encountered including *Scopulariopsis* spp., *Alternaria* spp. and *fusarium* spp.

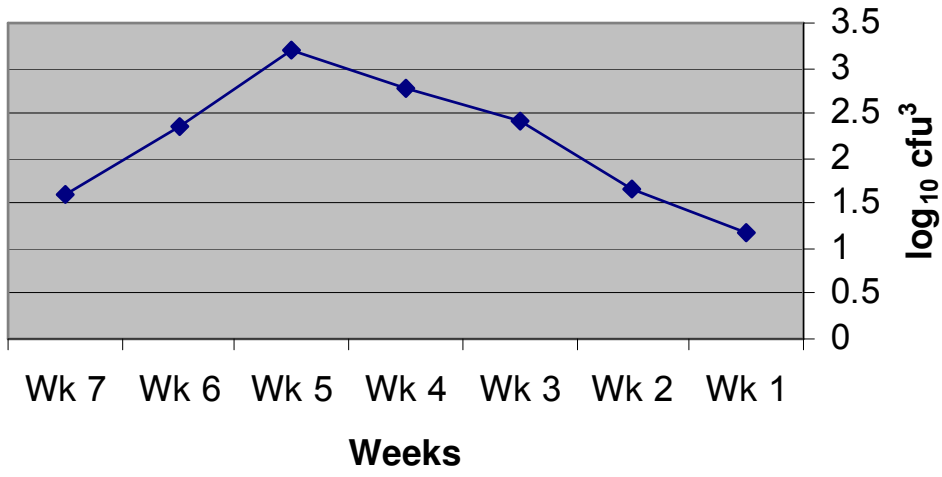

Fig. (1): Microbial contamination of air samples

Table (1): Means of the enumerated microbial groups or specis (log_{10} cfu/m^3) recovered from air samples in a broiler house.

Microbial group or species	Initial concentration	Maximum concentration	Mean
Aerobic bacteria	2.49	5.85	4.17
Total coliform	0.77	2.96	1.86
Esherichia coli	0.00	0.89	0.89
Fecal enterococci	0.79	2.39	1.59
Staphylococci	1.85	3.72	2.78
Pseudomonads	1.12	3.21	2.16
Yeast and molds	1.46	3.72	2.59

Table (2): Means of the enumerated microbial groups or species (log10 CFU/g) recovered from chicken litter.

Microbial group or species	Initial concentration	Maximum concentration	Mean
Aerobic bacteria	3.83	7.82	5.82
Total coliform	1.39	4.73	3.06
Escherichia coli	0.00	2.85	2.85
Fecal enterococci	0.00	4.30	4.30
Staphylococci	2.26	5.7	3.98
Pseudomonads	3.10	5.14	4.12
Yeast and molds	2.02	5.58	3.8

Table (3): Means of the enumerated microbial groupsspecies (log10 CFU/g) recovered from chicken feed.

Microbial group or species	Initial concentration	Maximum concentration	Mean
Aerobic bacteria	2.8	4.3	3.55
Total coliform	1.9	3.8	2.85
Esherichia coli	0.17	2.56	1.53
Fecal enterococci	0.00	1.98	1.98
Staphylococci	1.28	2.5	1.89
Pseudomonads	1.10	2.41	1.75
Yeast and molds	2.02	2.40	2.21

Table (4): The most probable number values (MPN) of total coliforms, *E. coli* and fecal enterococci / 100 ml water from drinkers in broiler house.

Coliforms	*E. coli*	Fecal enterococci
440	90	70
900	400	93
1500	390	75
1900	400	150
2400	930	210

Table (5): Pathogenic or potentially pathogenic bacterial species recovered from contaminated environmental samples in broiler house.

Bacterial species	No. of isolates	% of 96
E. coli	11	11.4
Enterobacter cloacae	3	3.1
Citrobacter freundii	2	2.1
Enterococcus fecalis	14	14.6
Klebsiella pneumonae	2	2.1
Staphylococcus aureus	9	9.4
Staphylococcus intermedius	2	2.1
Staphylococcus epidermidis	13	13.5
Pseudomonas aeruginosa	11	11.4
Pseudomonas fluorescence	2	2.1

Table (7): Results of mycological examination of environmental samples taken from broiler house.

Species	No. of isolates	% of 96
Absidia corymbifera	1	1.0
Alternaria alternata	2	2.1
Aspergillus flavus	26	27.1
Aspergilus fumigatus	3	3.1
Aspergillus niger	19	19.8
Aspergillus egyptiacus	1	1.0
Cladosporium herbarum	9	9.4
Fusarium moniliforme	4	4.2
Penicillium chrysogenum	11	11.4
Scopulariopsis brevicaulis	7	14.6
Candida albicans	13	9.4

DISCUSSION

The intensive poultry breeding requires high nutritional requirements and proper environmental conditions. Air quality and safe environments are an important consideration for animal houses and surrounding livings. Consequences of poor air quality include decreased production performance and poor bird health.

Numerous investigators have assessed the microbiological status of air pollution on poultry farms and its impacts on poultry health and productivity and stated that air concentration of microorganisms in poultry housing was greatly varies, which could in part be ascribed to different methods of sampling used in previous studies. The concentration of airborne microorganisms in layer housing to range from 360 to 3 781 cfu/l air ***Hartung (1994) and Müller (1987)*** from 17 to 5 860 cfu/l air. In the extensive study carried out by ***Seedorf et al. (1998)***, total airborne microorganism concentration in animal housing, expressed as a logarithm, was about 9.5 log cfu/h per 500 kg body weight (b.w.) in broiler houses. ***Northcutt et al. (2004)*** reported total aerobic bacteria $\log_{10}$ counts of 6.4 and 5.5 to 5.9 for broiler and hen houses, respectively. Meanwhile in Egypt, ***Sotohy (1989); Draz and Samaha (1992); Byomi and Trabees (2006)*** also detected airborne microorganisms in broiler and hen houses air in cfu/l. The Coliforms particularly, *E. coli*, and fecal enterococci that are part of intestinal flora of man and animals and consider as a bioindicator of pollution. Hojovec et al. (1977) evaluated the air quality in poultry house using *E. coli* as an indicator. Our results revealed coliforms and *E. coli* were detected at a mean values of 1.86 and 0.89 $\log_{10}$ in air of the examined house, respectively. Past investigation revealed that the ambient air of animal

houses is often polluted with airborne *E. coli* ***(Zucker et al., 2000; Duan et al. 2006)***. The obtained results were relatively similar to those reported by ***Draz and Samaha(1992)and El Zarka (2003).Northcutt et al. (2004)*** found that coliform counts were highest in air sampled in the hen house (2.5 $\log_{10}$ cfu/ml). The coliforms and *E. coli* concentrations in air of poultry houses are correlated to their concentration in litter, feeds, biological activity of birds and stocking density ***(Hojovec and Fisher, 1986; Lovett et al., 1971)***. Moreover, the other fecal indicator, fecal enterococci, was reported at a mean value of 1.59 $\log_{10}$ in air. This was relatively similar with the previously reported fecal enterococci in the air of hen houses by ***El-Zarka (2003); Byomi and Trabees (2006).*** The plate counts of *Enterococcus* spp. appeared to reflect their relative abundance among chickens normal flora of gastrointestinal tract ***(Zhu et al., 2002).*** Counts for Staphylococci and Pseudomonads were highest in the house (2.78 and 2.16 $\log_{10}$, respectively). Our results were in coincided with the past investigations that detected relatively higher prevalence of Staphylococci and Pseudomonads in poultry houses ***(Northcutt et al., 2004; Byomi and Trabees, 2006).***

The mycological investigation of air in the house revealed total fungal count at a mean of 2.59 $\log_{10}$ and *Aspergillus* in addition to yeasts of genus *Candida* were the predominant genera encountered. ***Northcutt et al. (2004) and Fulleringer et al. (2006)*** detected mold and yeast count in a higher concentration in air of poultry house. In Egypt, Past investigations revealed higher prevalence of fungi in the ambient air of poultry houses as *Aspergillus spp*., *Penicillium spp*., *Cladosporium spp*., *Scopulariopsis spp*., *Absidia spp*., in addition to yeasts of genus *Candida* were the predominant genera encountered ***(Swelim et al., 1993; Moawed et al., 1995)***.

The results revealed that the air concentration of microorganisms increased with the bird growth and age whereas low at the beginning and end of fattening period, which was attributed to limited birds activity. Similar conclusions were also reported by ***Saleh et al. (2005) and Vucemilo et al. (2007)*** investigating the effect of fattening poultry age and season on the bioaerosol concentration in poultry houses. Furthermore, ***Sauter et al. (1981)*** reported that the airborne microflora increased with the density housing of birds.

Other factors influence the air concentration of microorganisms. The microbiological evaluation of litter, feed and water from the house were detected to be contaminated with the microbial groups or species analyzed. In poultry industry, the use of feed and water with adequate microbiological quality, it is of fundamental importance. Since many birds have access to the same water and feed sources, quality problems will affect a great number of animals. ***Fulleringer et al. (2006)*** reported constant fungal contamination of litter and poultry feed through out the surveillance periods. ***Byomi and Trabees (2006)*** revealed massive contamination of water sources with fecal enterococci and coliforms. ***Arotupin et al. (2007)*** recorded highest bacterial and fungal counts in commercial poultry feeds. Litter is a major factor because the population of microorganisms varies depending on the dynamics of litter exchange ***(Lu et al., 2003; Vucemilo et al., 2007)***.

The bacterial and fungi genera detected in the present study were consistent with literature data ***(Sweilem et al., 1993; Hartung, 1994; Moawed et al., 1995; Seedorf et al., 1998; Baykov and Stoyanov, 1999; Szejniuk and Kuczek, 2000; Radon et al., 2002; Bakutis et al., 2004;***

Byomi and Trabees, 2006; Fulleringer et al., 2006). The different bacterial and fungal species that were isolated during the study period are considered pathogenic and or potentially pathogenic for chickens. *S. aureus* is a common cause of synovitis and arthritis which is a septicemic staphylococcal infection localized in the joints and tendon sheaths leading to bumble foot ***(Devries et al., 1975)***. Coli-group bacteria and *E. coli* are known to be a diarrheic agents. Furthermore, *Aspergillus flavus*, *Aspergillus fumigatus* and *Candida albicans* are causes of avian aspergillosis and candidosis.

In conclusions, This study was contributing to the understanding of the level and composition of environmental pollutants in poultry house. It could be concluded that analysis of the microbial contamination of a poultry house is necessary for taking suitable hygienic measure to improve bird health performance, human health and surrounding environment as well.

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تحليل التلوث البيئي بالبكتيريا والفطريات في احدي بيوت (حظائر) بداري التسمين بمحافظة قنا – مصر

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ترتبط صحة الطيور بدرجة كبيرة بجودة البيئة المحيطة بالطائر. تم دراسة الحالة الميكروبيولوجية في بيئة احدي حظائر بداري التسمين بمحافظة قنا – مصر. كانت المعايير المختبرة هي العد البكتيري الكلي، العد الكلي للفطريات (خمائر و أعفان)، عد الكوليفورم الكلية وميكروب إيشيرشيا القولون، الميكروب السبحي البرازي، المكورات العنقودية و البكتيريا من جنس الزائفات (السيدوموناس). تم تجميع عينات من بيئة الطيور أسبوعيا خلال فترة التسمين، اشتملت علي عينات من علائق التسمين، فرشة الطيور، مياه السقايات و الهواء. تم عزل وتعريف البكتيريا والفطريات من العينات البيئية المختلفة باستخدام الأوساط الغذائية التفريقية و الانتقائية المناسبة وكذلك الاختبارات البيوكميائية. أظهرت النتائج بوضع عام إلي حدوث تلوث في بيئة الطيور، ازداد مع نمو وكبر الطيور في العمر. كان متوسط عدد المعايير المختبرة في هواء الحظائر (عدد لوغارثمي) كالتالي: العد البكتيري الكلي 4.17 ، العد الفطري الكلي 2.59 ، عدد الكوليفورم الكلية 1.86 ، بكتيريا إيشيرشيا القولون 0.89 ، الميكروب السبحي البرازي 1.59 ، المكورات العنقودية 2.78 ، عدد البكتيريا من جنس الزائفات (السيدوموناس) 2.16. أظهرت العوامل البيئية الأخرى مثل علائق الطيور، فرشة الطيور و مياه السقايات، تلوثا ميكروبيا بواحد أو أكثر من المعايير المختبرة. أظهر الفحص البكتريولوجي للعينات البيئية المختلفة إلي شيوع عزل البكتيريا من جنس المكورات العنقودية، الزائفات وبعض بكتيريا مجموعة القولون، في حين أظهرات نتائج الفحص الميكولوجي إلي عزل جنس الرشاشات ، البنسليوم وكذلك الخمائر المبيضة بصورة سائدة. خلصت الدراسة إلي وجود تلوث في البيئة المحيطة بالطيور، مما له الأثر في نمو وصحة الطيور، حيث أن معدلات النمو الجيدة تكون دائما مرتبطة ببيئة جيدة وخالية من الميكروبات. أوصت الدراسة إلي اتخاذ إجراءات صحية مثل التطهير وغيرة، من شأنها ضبط البيئة المحيطة بالطيور لوقاية الطيور من الإصابة ببعض الميكروبات وكذلك العاملين القائمين علي رعاية الطيور وتربيتها ومنع حدوث تلوث للبيئة الخارجية المحيطة بالحظائر.