Bacteriological, Mycological and Histopathological Studies On Zoo Birds Suffering from Respiratory Manifestations

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Summary

One hundred and eighty two samples were collected (60 dead birds and 122 nasal swabs from diseased birds) from El-Fayoum zoo, Fayoum governorate. These birds are belonging to 3 species (Mallard ducks "*Anas platyrhynchus*"; Guinea fowl "*Numida meleagris*" and Common Pea fowl "*Pavo cristatus communis*"). In this study, 119 bacterial isolates were isolated. The different bacterial species were *Pasteurella multocida; Salmonella typhimurium; E. coli and Staphylococcus aureus*. The highest incidence of bacterial isolation was observed in mallard (44.5%) followed by G.fowl (40.3%) then Pea fowl (15.1%). The most common bacterial species isolates from the examined birds were *P. multocida* (31.3%) followed by *Salm. typhimurium* (17.0 %) followed by *E.coli* (13.7%) and then *S. aureus*, (3.3%). Furthermore, 81 fungal isolates were isolated. The highest incidence was observed in Mallard (41.9%) followed by Guinea fowl (32.1%), then Pea fowl was the lowest incidence (25.9%). Suggested reasons and explanation of that have been discussed in details. *A. fumigatus* was the most prevalent (27.4%) followed by *A. flavus* (10.9%) *A. niger* (3.8%) and the lowest incidence was *C. albicans* (2.2%). 61.3% and 62.9% of the bacterial and mycotic isolates respectively were belonging to the nasal swab samples isolations, importance of nasal swabs use in wild birds investigation has been discussed.

Gross and histopathological lesions were seen in liver, lung, kidney, and heart. The pathlogical results in this study came to confirm those of bacteriological and mycotic studies. There were focal necrosis and infiltration of heterophils. There were hemorrhagic septicemia with widespread vascular damage and focal

necrosis in liver and spleen. Lung pathological lesions were consisted of fibrinous pneumonia, there was congestion and infiltration of inflammatory cells in the pulmonary tissues, granuloma consisted of fungal hyphae surrounded by inflammatory cells as a result of aspergillus infection, especially in Mallards.

Introduction

Respiratory problems among zoo birds are considered the most important and serious affections affecting them which may be brought on by lack of proper management, over rowdiness and competition for food. Additionally, the presence of birds' cages, premises and even the yards of birds close and near to each other facilitate the transmission of diseases among the birds. Also, animal keepers have great responsibility in spreading the infection during their daily routine work. Bacterial infection is considered one of the major reasons for disease leading to great losses among zoo birds as recorded by (1, 2 and 3). A lot of authors isolated many different bacterial species such as *Paseurella, Salmonella, E.coli* and *staphylococcus* (4, 5, 6, 7, 8 and 9).

Mycotic infection also, is considered as a strong cause for respiratory manifestation. Many authors isolated Aspergillus species (*A. fumigatus, A. flavus* and *A.niger*) and *Candida albicans* from respiratory affected zoo birds (10, 11, 12 and 13).

The aim of this work was to isolate and identify the bacterial and mycotic causes of respiratory problems which cause high morbidity and mortality among some zoo birds as well as to study the histopathological changes in different organs and also, to detect the efficiency of nasal swabs in estimating the healthy status and the incidence of infection in zoo birds as highly valuable birds.

Material and Methods

Samples

A total of 182 birds of different species in El-Fayoum zoo, Fayoum governorate, were examined (Table 1). Tissue specimens from lung and heart were taken from the affected freshly dead bird (60 in number). Nasal swabs were aseptically taken from the nasal clift of the respiratory affected birds (122 in number) and transferred in icebox directly to the wildlife laboratory with minimum of delay. All samples were subjected to bacteriological and mycological examinations.

*Media used

All media were obtained as dehydrated media; nutrient broth; nutrient agar; MacConkey broth; MacConkey agar; blood agar (5% sheep defibrinated blood);in addition to Sabouraud dextrose agar and selenite F-broth (Difco). The media were prepared according to the media producer and (14 and 15).

Bacterial Examination:

Isolation and identification of bacterial isolates were done as proposed by (16) as follows:

1-Salmonella and E coli were identified using Oxidase test; growth onto triple sugar medium; indole production; urease activity into Christensen's urea medium; methyl red and V.P. test; sugar fermentation tests. All biochemical reactions were recorded finally at least five days post incubation at 37 ˚C.

2- Pasteurella species were identified according to (16) using Growth onto Maonkey agar's media plates; motility test; indole production tesy; oxidase test; catalse test; H2S production test; nitrate reduction test, and sugar fermentation tests.

3- Staphylococcus species were identified according to (16) using, coagulase test; nitrate reduction test; urease test and sugar fermentation tests. All the suspected isolates were subjected to serological typing by slide agglutination test using standard polyvalent and monovalent antisera according to (17).

Mycotic Examination:

Isolation and identification of fungi was carried out according to (18 and 19) by careful observation of the macroscopic and microscopic characterization of the mould colonies on the selected media. For yeast identification, Rice agar was used. Serotyping for the identified isolates has been done in the Dept. of serology, Animal Health Research Institute, Dokki.

Histopathological Examination:

Specimens from liver, kidney, spleen, lung, heart and brain were collected and fixed in 10% neutral buffered formalin for histopathological examination. After fixation all specimens were taken, dehydrated in graded alcohol, embedded in paraffin. Five microns sections were obtained and stained with routine Hematoxylin and Eosin stain (H&E) as described by (20).

Table (1): Total number of examined zoo birds

	Bird species			Total
	Malllard	Guinea Fowl	Pea Fowl	
Nasal swabs	47	54	21	122
Bird specimens	27	24	9	60
Total	74	78	30	182

RESULTS

Clinical signs of the examined birds showed, weakness, poor appetite, lusterless feather, depression, coughing and purulent nasal discharge. Postmortem of the freshly dead birds showed purulent exudates in the nasal passage, some nodules in the lungs, air sacs and sinuses. Enteritis, hepatitis, pericarditis and septicemia in liver and kidney. The results of bacterial examination in both bird specimens and nasal swabs are shown in tables 2 and 3, in which, 119 bacterial isolates were isolated from 182 samples. 73 bacterial isolates have been isolated from the nasal swabs samples (61.3%)

and 46 isolates (38.6%) of the isolates have been isolated from the bird specimens. . In case of the fungal isolation, 81 fungal isolates have been detected of which 51 isolates were detected from the nasal swabs (62.9%) and 30 isolates (37.0%) were detected from the bird specimens**.**

Table (2): Number and percentage of isolated bacteria species from both the bird specimens and nasal swabs of different examined zoo birds.

	Mallard (74)			G.Fowl (78)			P.Fowl (30)			Total (182)		
	sp	sw	total	sp	Sw	total	sp	sw	total	sp	sw	Total
P.multocida - %	10	16	26 35.1	8	13	21 26.9	4	6	10 33.3	22	35	57 31.3
S.typh - %	7	9	16 21.6	5	8	13 16.6	1	1	2 6.6	13	18	31 17.0
E.coli - %	2	7	9 12.1	5	8	13 16.6	1	2	3 10.0	8	17	25 13.7
St.aureus - %	1	1	2 2.7	1	0	1 1.3	1	2	3 10.0	3	3	6 3.3
Total - %			53 44.5			48 40.3			18 15.1			119

Table (3) Number and percentage of isolated fungus species from both the bird specimens and nasal swabs of different examined zoo birds**.**

	Mallard (74)			G.Fowl (78)			P.Fowl (30)			Total (182)		
	sp	sw	total	sp	sw	total	sp	sw	total	sp	sw	Total
A.fumigatus - %	6	10	16 21.6	8	14	22 24.3	3	9	12 40.0	17	33	50 27.4
A.flavus - %	4	7	11 14.8	0	4	4 3.8	2	3	5 16.6	6	13	20 10.9
A.niger - %	3	4	7 9.4	0	0	0 00.0	0	0	0 00.0	3	4	7 3.8
C.albicans - %	0	0	0 00.0	0	0	0 00.0	4	0	4 13.3	4	0	4 2.2
Total - %			34 41.9			26 32.1			21 25.9			81

Gross and histopathological changes

Mallard

Examined birds showed different lesions. The main pathological lesions were in lungs, liver and spleen. Lungs were congested and had focal hepatized areas. Liver was enlarged and had peticheal hemorrhages and focal necrosis while the spleen was enlarged. No changes were recorded in heart and kidneys. The microscopical examination, showed congestion of pulmonary and perialveolar blood vessel and perivascular edema. Edema was diffuse in pulmonary tissues (Fig.1). Some cases showed diffuse pneumonia which is characteristic to pasteurellosis, pneumonic foci were composed of fibrinous exudates, necrosis and heterophilic infiltrations (Fig.).Liver showed focal areas of coagulative necrosis along with fatty change. The fat globules showed positive reactions to oil red o stain (Figs 3 & 4). Some cases had hyperplasia of bile ducts and diffuse mononuclear cell infiltration (Fig 5). Spleen showed depletion and hyperplasia of reticulo-endothelial cells (Fig. 6).

Guinea Fowl

Lesions were severe and characterized by adhesive pericarditis and fibrinous perihepatitis with necrotic foci in liver, lung and kidneys. Some cases showed septecimia characterized by petecheal hemorrhages in serosal membranes, epicardium, liver and kidneys. Microscopically, liver showed different varities of lesions that consist of focal aggregation of heterophils, degenerations and necrosis along with leucocytic infiltration (Figs.7&8). Perihepatitis was characterized by fibrinous exudates and heterophile infiltrations (Fig. 9). Diffuse pneumonia characterized by infiltrations of heterophils and mononuclear cells was observed (Fig. 10). Pericarditis is characteristic to collibacillosis and formed from serofibrinous exudates, heterophils, macrophages and lymhpocytic infiltrations (Fig.11). Heart muscles showed degeneration and vacuoles of different sizes and shape with leucocytic infiltrations (Fig. 12). Kidneys showed congestion and hemorrhages of peritubular blood vessels (Fig. 13).

Pea Fowl

Gross examination showed adhesive pericarditis, coagulative necrosis in liver and enlarged spleen and focal hepatization in lung. Microscopically, lung lesions showed acute pneumonia characterized by congestion of pulmonary blood vessels, leucocyte infiltrations and diffuse edema (Fig.14). Some cases showed chronic pneumonia characterized by focal aggregations of chronic inflammatory cells mainly macrophages and lymphocytes (Fig.15). Liver showed severe congestion, diffuse vacuolar degeneration and necrosis (Figs. 16, 17 & 18).

Aspergillosis

Grossly, the birds showed large grayish nodular areas of consolidation, thickening of the wall of air sac, white moldy growth on the surface of lung. Microscopically, the nodules consisted of coagulative necrotic center in which colonies of the radiating hyphae are present. The inflammatory reaction consists of macrophages, epithelioid cells, multinucleated giant cells as well as lymphocytes and fibroblasts (figs. 19, 20, 21 &22).

Discussion

Clinical signs and postmortem findings of the examined birds were in agreement with that recorded by (12, 21 and 9) In the current study, a total of 119 bacterial isolates from 182 examined birds have been isolated as shown in table (2). Bird species variability to catch the infection and the distribution of the isolated bacteria in those birds will be discussed as follows:-

Fig (1): Lung, showing ,congestion of pulmonary, perialveolar blood vessel and diffuse edema pulmonary tissues. (H&E stain. X 40).

Fig.(2): Lung, showing diffuse pneumonia, composed of fibrinous exudates, necrosis and heterophilic infiltrations. (H&E stain. X 10).

Fig (3): liver, showing coagulative necrosis along with fatty change. (H&E stain. X 20).

Fig (4): liver, showing the red stained fat globules. (Oil red o stain. X 40).

Fig (5): liver, showing hyperplasia of bile ducts and diffuse mononuclear cell infiltration (H&E stain. X 20).

Fig.(6): Spleen, showing depletion and hyperplasia of R.E.Cs. (H&E stain. X 20).

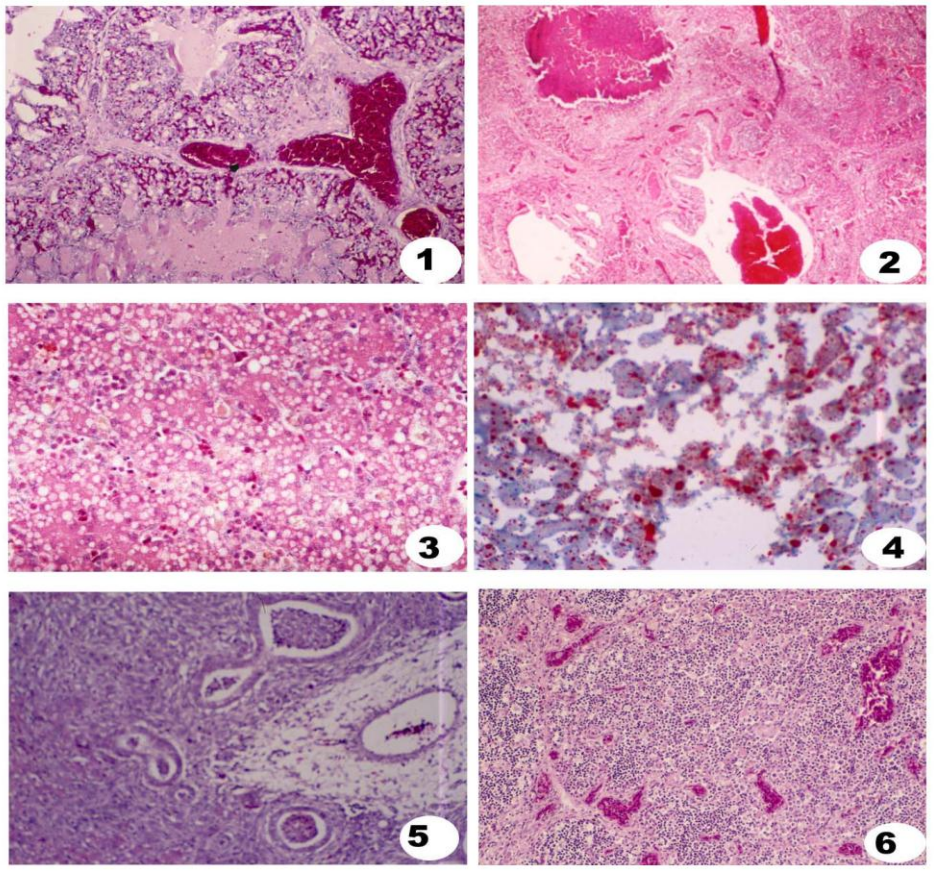


Fig (7): liver, showing focal aggregation of heterophils. H&E stain. X 40.
Fig (8): liver, showing degenerations and necrosis along with leucocytes infiltration. (H&E stain. X 40).
Fig (9): liver, showing Perihepatitis characterized by fibrinous exudates and heterophils infiltration. .
Fig (10): lung, showing diffuse pneumonia characterized by infiltrations of heterophils, fibrinous exudates and mononuclear cells. (H&E stain. X 10).
Fig (11): heart showing pericarditis, formed from serofibrinous exudates, heterophils, macrophages and lymphocyte infiltrations. (H&E stain. X 40).
Fig (12): heart muscles showing degeneration and vacuoles of different sizes and shape with leucocytes infiltrations. (H&E stain. X 40).

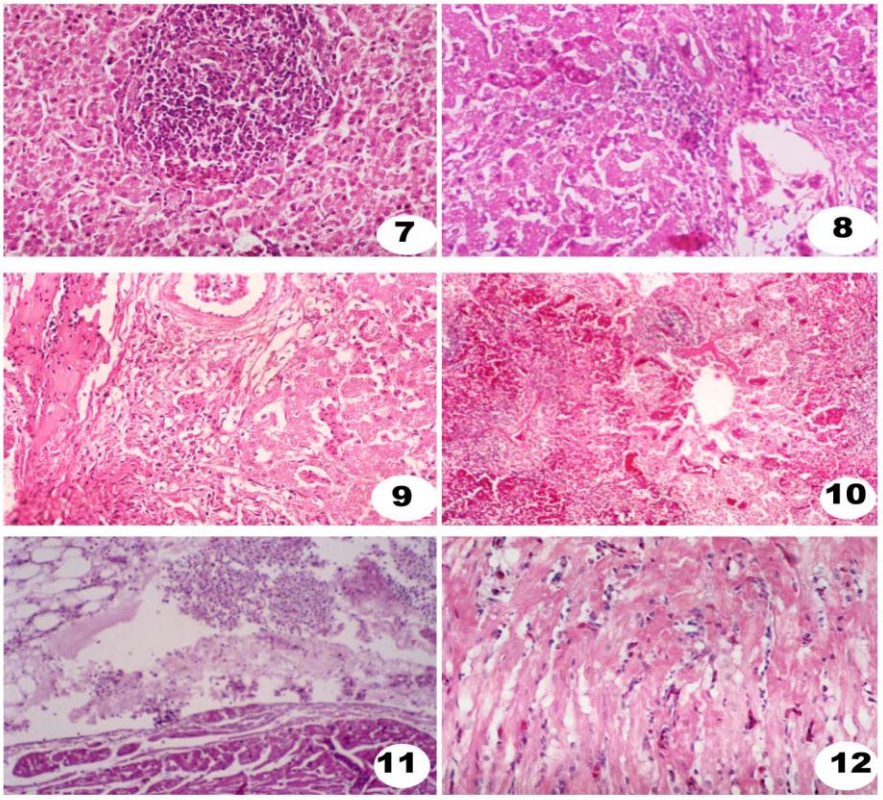


Fig. (13): Kidneys showing congestion and hemorrhages of peritubular blood vessels. (H&E X 20).

Fig. (14): lung showing congestion of pulmonary blood vessels, leucocytes infiltrations and diffuse edema. (H&E X 40).

Fig. (15): lung showing chronic pneumonia characterized by focal aggregations of macrophages and lymphocytes. (H&E X 20).

Fig. (16): Liver showing severe congestion. (H&E X 20)

Fig. (17): Liver showing diffuse vacuolar degeneration. (H&E X 20)

Fig. (18): Liver showing focal coagulative necrosis along with fatty change. (H&E. X 20).

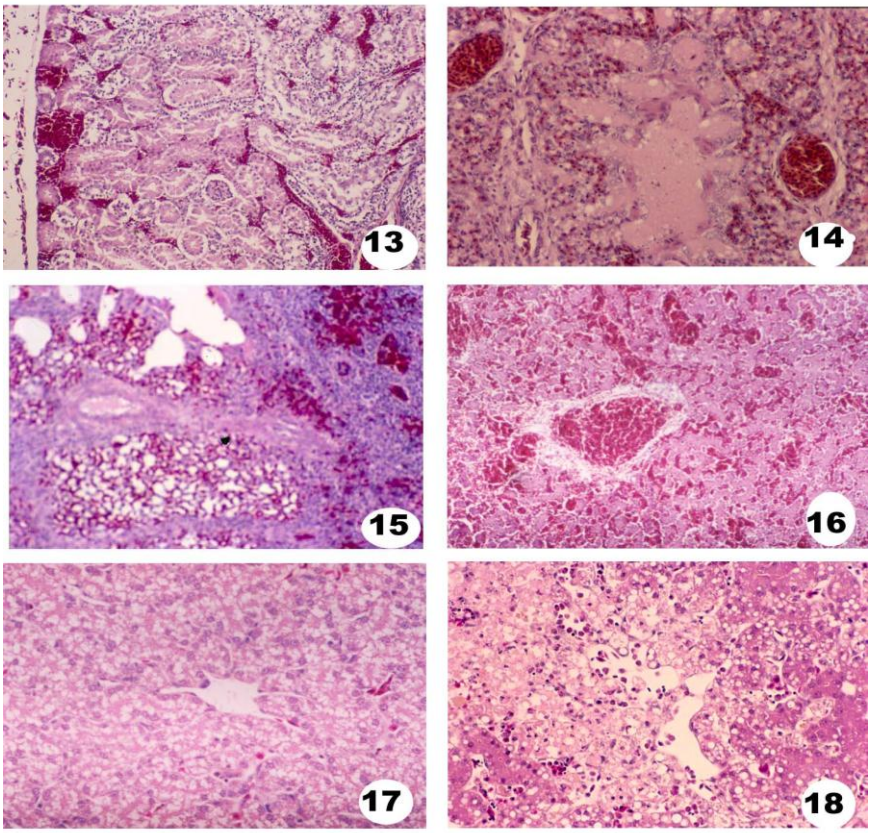
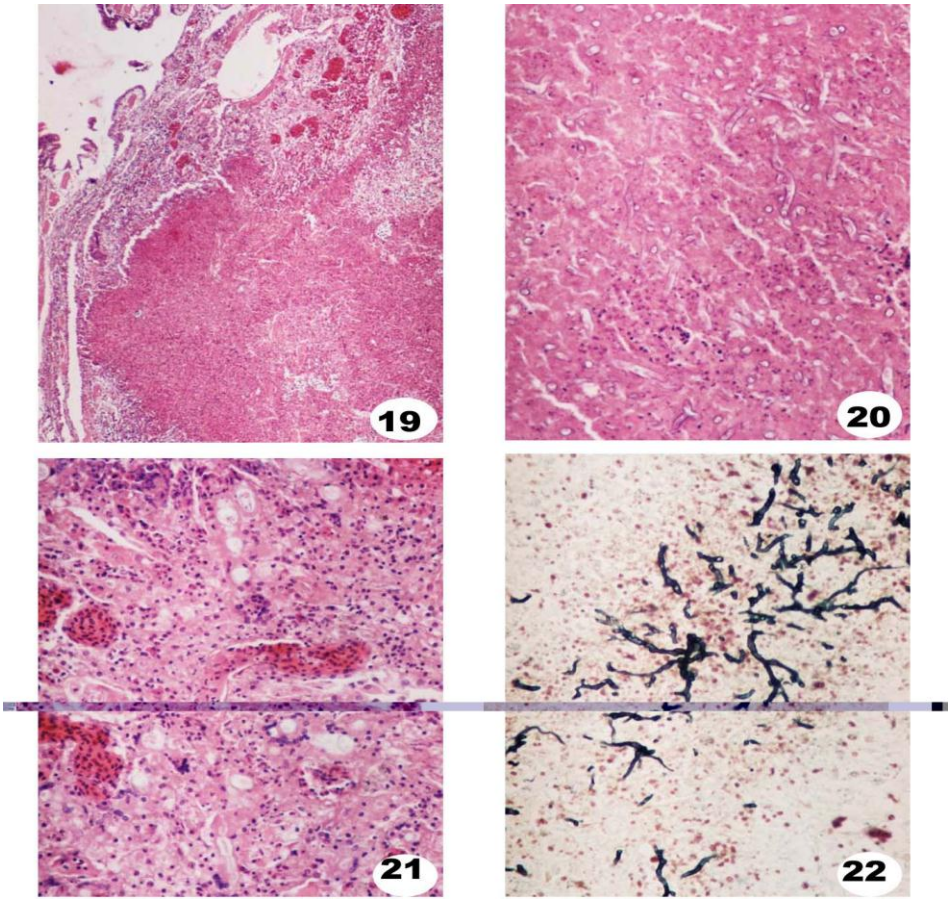


Fig.(19): Lung, Aspergillosis showing granuloma formation with caseated center and mononuclear cell infiltration. X 20.

Fig.(20): Lung, Aspergillosis, higher magnification of the granuloma showing the eosinophilic septated hyphae. H&E. X 40.

Fig.(21): Lung, Aspergillosis, higher magnification of the granuloma showing many spores and mutinucleated giant cells engulfed spores. H&E. X 40

Fig.(22): Lung, Aspergillosis, higher magnification of the granuloma showing the black stained septated hyphae. Grocut's stain. X 40.



Bird species variability to catch the infection

The highest incidence of bacterial isolation was observed in mallard (44.5%). The highest bacterial isolate was P. multocida (35.1%). These results come in consistance with those reported by (4) who isolated this strain in a percentage of 36.4% from water fowl in Giza zoo and agreed with (22) who confirmed that, P. multocida was highly obtained from mallard, and nearly close to that obtained (37.9%) by (23), They come in agreement with (24) who stated that, the main cause of mortality in mallard in different states of America was from P. multocida. These results also agreed with (7 and 25) who found that a broader spectrum of wild bird species have been killed from (1907) till (1999) by avian cholera but waterfowl have suffered the greatest losses.

The other bacterial isolates were as follow: - Salm. typhimurium (21.6%) then E.coli (12.1%) then S. areus (2.7%). These results are in complete agreement with those of (26 and 23) who isolated same bacterial species from the same bird species in nearly similar percentage 24.0%, 14.7% and 2.3% (in S. typhimurium, E.coli and S.aureus respectively) .

G.fowl was the second higher incidence (40.3%) of infection after mallard, where these results are in agreement with (5). P.multocida was the highest bacterial isolate (26.9%), Sal. typhimurium and E.coli were isolated in the same percentage (16.6%) while S.areus was the lowest incidence (1.3%), these results were similar to those obtained by (23) who isolated Sal. typhimurium and E. coli in a percentage of 17% and 14% respectively, and come in agreement with (27), meanwhile they disagree with (13) who isolated E. coli in a higher percentage than Pastreulella .

Pea fowl was the lowest of the examined birds (15.1%), this result goes hand in hand with that reported by (28 and 23) who isolated bacterial incidence in P. fowl less than that in G. fowl. Percentage of isolates in P. fowl of different bacterial isolates (Pastreulella, Salmonella, E.coli, and Staphylococcus) was as follows, (33.3%, 6.6%, 10.0% and 10.0%respectively) where Pastreuella was the highest isolates, these results agreed with (29 and 23) and little differ with (5) who isolated Pastreulla as the

second higher bacteria from the same species and E.coli was the highest. P. multocida was mostly obtained from mallard (35.1%) followed by P. fowl (33.1%) then G. fowl (26.9%), these findings are agreed with those obtained by (13). Salm. typhimurium was mostly isolated from mallard (21.6%) followed by G. fowl (16.6%) then P.fowl (6.6%). These findings are similar to the obtained results by (30, 5 and 6).

E coli was mostly isolated from G. fowl (16.6%) followed by mallard (12.1%) then followed by P. fowl (10.0%). These findings are parallel to those obtained by (23 and 13). S.aureus was mostly isolated from P. fowl (10%) followed by mallard (2.7%) and the lowest incidence was in G. fowl (1.3%), same prevalence have been obtained by (23).

Distribution of bacterial species

In the present study (Table 2), the most common bacterial species isolates from the examined birds, which may be the main cause of respiratory tract affections, was P. multocida (non-haemolytic) serotype A:3, where the percentage of isolation from all examined birds was 31.3%., this result is little higher than that reported by (31) who isolated P. multocida in 24.7% among different zoo birds. Salm. typhimurium, isolated in an incidence of 17.0 % from the total examined birds and it occupied the second higher bacterial isolates after Pastreuella.

In this study, it is evident that Salm. typhimurium infection considered one of the most important causes of respiratory affections followed in its importance to pasteurella infection. This conclusion agreed with that recorded by (32, 4, 33, 13, 34 and 35) who reported that, salmonella species were mainly found in zoo birds and cause respiratory troubles. Moreover, they confirmed that Salm. Typhimurium is the most common serotypes.

E.coli was isolated in a rate of 13.7% from the total examined birds and S. aureus, was isolated in an incidence of 3.3% from the examined birds. These results are in consistance with that of (36) and agreed with (23) who isolated S. aureus from the same species in a percentage of 5.4%.

From these results it could be concluded that Pasteurella, Salmonella and E.coli could be considered the most serious bacterial infection in zoo birds.

Regarding to the fungal isolates, as shown in table (3) 81 fungal isolates were isolated from 182 samples. The highest incidence was observed in Mallard (41.9%) followed by Guinea fowl (32.1%), then Pea fowl was the lowest incidence (25.9%). These results are in consistance with those isolated by (1, 10 and 37). Meanwhile, they are in contrary with those obtained by (13) who isolated the same fungal species from Pea fowl in higher rate followed in Guinea fowl and then in mallard. Concerning the distribution of different fungal species, the current results proved that A. fumigatus was the most prevalent (27.4%) followed by A. flavus (10.9%) followed by A. niger (3.8%) and the lowest incidence was in the side of C. albicans (2.2%). These results are nearly similar to those obtained by (13) who isolated these fungal species from the same examined bird species in the same prevalence. These results are in agreement with those isolated by (37, 12 and 38) who isolated same Aspergillus species from ducks. From these results it is clear that C. albicans was isolated only from Peafowl (13.3%) in a percentage nearly as those isolated from same species by (11).

Mallard was the highest bird in mycotic infection (41.9%) and this may be referred to the bad hygienic conditions in the zoo, where wet areas, wet food and the full absence of drainage system in the water pool where the mallard live, an area like this is the best media for the fungal surviving, similar findings have been reported by (39). Highly polluted water is detrimental to the ducks health and can affect overall performance as was confirmed by (40).

Bacterial and mycotic infection prevalence in p. fowl and G. fowl was lower than that in case of mallard. The incidence proved a higher rate in G. fowl (32.1%) more than that in P. fowl (25.9%), this may return to that , P. fowl is the showiest bird in the zoo, and the reflect of that is the great care of this bird regarding the yard, bedding area, and food supplying. In contrary, G. fowl doesn't meet

this situation, it is suffering from unhygienic conditions regarding food, cages and yards, in addition to the over supplied food from the visitors that may be contaminated and all these factors have bad affect on its health in general.

As it is shown in tables (2) and (3), the bacterial incidence and mycotic incidences were higher in the swab isolates than in case of bird specimens. 119 bacterial isolates have been isolated from the all examined samples, 73 bacterial isolates have been isolated from the nasal swabs sample representing 61.3% and the rest (46) isolates were representing 38.6% of the specimen's isolates. In case of the fungal isolation, 81 fungal isolates have been detected in which 51 isolates were detected from the nasal swabs representing (62.9%) and the rest of isolates (30) that representing 37.0% was specimen's samples. From these results we concluded that, nasal swab samples collected from the examined birds can be used as a tool for detection of the bacterial and mycotic infection among zoo and wild birds, a procedure that provide an excellent data upon the birds without scarifying of these highly valuable birds. This was in full agreement with (4) who isolated p. multocida, S. typhimurium, E. coli and s. areus from the nasal swabs of mallard and domestic ducks. It also agreed with (41) who used the nasal swabs as the only source for detection of the nasal flora in Peregrine falcons. This conclusion comes in agreement with (8) who detected the same bacterial and fungal species that have been isolated in this study from the nose of lanner falcons. Similar results of the current study have been recorded by (9) who used nasal swabs from diseased captured birds and even from recently dead birds for detection of P. multocida in Mallards.

The pathlogical results in this study came to confirm those of bacteriological and mycotic studies, Mallard had the highest percentage of p. multocida infection and the pathological lesions were either acute or chronic lesions. Ducks that died acutely of avian cholera had lesions of a hemorrhagic septicemia with widespread vascular damage and focal necrosis in liver and spleen. Lung hitopathological lesions are consisted of fibrinous pneumonia, and these came in accordance with (41). Our results came in

accordance also with that of (42, 43, 45 and 9). Guinea fowl and Pea fowl showed a verities of lung lesions including congestion with infiltration of inflammatory cells that are attributed to pasteurella, Salmonella, and E. coli infections.

Hepatitis was seen in this study, in all examined birds. There were focal necrosis and infiltration of heterophils, this is due to a long standing infection, this finding recorded by (46). Such lesions were associated with Salmonellosis, pasteurellosis and coliseptecemia, perihepatitis associated commonly with pasteurella and E. coli infection and our findings came in accordance with those of (45).Mallard also showed the highest percentage of fungal isolation, from the pathologicl point of view, there was congestion and infiltration of inflammatory cells in the pulmonary tissues, granuloma consisted of fungal hyphae sounded with inflammatory cells as a result of aspergillus infection, similar results were recoded by (12 and 48). The described lesions were in accordance with those of (49).

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دراسات بكتريولوجية وميكولوجية وباثولوجية علي الطيور المصابه بأعراض تنفسيه في حديقة الحيوان

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تم أخذ عدد 182 عينه طائر بري (60 من طيور نافقة و122 مسحة من الأنف للطيور المريضة) من حديقة الحيوان بالفيوم. هذه الطيور من فصائل مختلفة تشمل البط الخضاري ودجاج الوادي والطاووس الهندي. تم عزل 119 معزول بكتيري وأهم هذه المعزولات باستيريللا مالتوسيدا A3 والسالمونيللا تايفيموريوم والأشيريشيا كوللاي والمكور العنقودي الذهبي. النسبة الأعلي للبكتيريا المعزولة كانت من نصيب طائر البط الخضاري (44.5%) يليها دجاج الوادي (40.3%) ثم يليها الطاووس (15.1%).أعلي نسبة من البكتيريا المعزولة كانت للباستييريللا ((31.3%) يليها السالمونيللا (17%) يليها الاشريشيا كوللاي (13.7%) ثم المكور العنقودي. (3.3%) . أيضا تم عزل 81 فطر. النسبة الأعلي كانت في البط الخضاري (41.9%) يليها دجاج الوادي (32.1 %) ثم يليها الطاووس (25.9%). أسباب وتفسير حدوث هذه الاصابات في الطيور المختلفه بنسب متفاوتة تم مناقشتها بالتفصيل. الأسبريجيللاس فيوميجاتس (27.4%) يليها الاسبيريجيللاس فلافاس (10.9%) يليها الأسبيرجيللاس نايجر (3.8%) ثم الكانديدا البكانس (2.2%) . 61.3 % و 62.9% من المعزولات البكتيرية والفطرية بالترتيب كانت معزولة من مسحات الأنف. أهميه استخدام المسحات في فحص الطيور البرية نظرا" لقيمتها البيئية والبيولوجية تم مناقشتها. ظهر العديد من التغييرات الهستوباثولوجية في الكبد والرئة والكليتين والقلب. أتت هذه التغيرات لتأكد نتيجة الفحص البكتيري والميكولوجي. ظهرنتائج العديد من التسمم الدموي والاحتقانات وظهرت تغيرات تحطمية في خلايا الكبد. أظهرت الرئتين التهابا رئويا واحتقانات ووجود خلايا التهابية في النسيج الرئوي.. وفي رئة البط الخضاري بصفة خاصة ظهرت بنسبة عالية خيوط فطرية مقسمة كنتيجة للاصابة بالاسبيرجيللاس.