Histopathological studies on the effect of mycotic infection on reproductive system in bulls.

By

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Summary

Reproductive organs (testes, epididymis), lung, liver, intestine and kidney specimens of 100 slaughtered bulls were collected from Cairo and Giza abattoirs during the period from April to October 2007. All samples were subjected to mycological and pathological examinations. The results of mycological examination revealed that the, *Aspergillus flavus* was the predominant toxigenic fungi isolated from all the specimens. In addition *A. achracus*, *Fusarium species* and *Pencillium species* were also isolated. The toxin extracted were aflatoxin, ochratoxin, zearalenone and penicillic acid. Microscopical examination of the testicular tissue specimens revealed, depletion of spermatogonial cells. Some seminiferous tubules either showed desquamation of the lining cells with the formation of cellular casts with edema and mononuclear cells infiltration of the interstitial tissue or appeared necrotic with complete destruction of sertoli and germinal cells. Lesions of the tubules of epididymis were recorded. The main fungi isolated were, *Aspergillus spp.* Microscopical examination of the lung tissue specimens revealed 3 types of pneumonia hemorrhagic pneumonia, granulamotous pneumonia and bronchopneumonia. The most prevalent fungal isolates of were *Aspergillus spp.*, *Pencillium spp.*, *Fusarium species*, *Mucor spp.* and *Candida spp.* Multiple sporadic necrotized hepatocytes were noticed scattered throughout the hepatic parenchyma. The intestine and kidney were also affected. It is concluded that the reproductive system of bulls was badly affected with fungal infection rather than other organ of the body.

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INTRODUCTION

Mycotoxins are secondary fungal metabolites contaminate various feed and food commodities due to global occurrence of toxigenic molds, consequently mycotoxins containing foods have been formed all over the world, the extension of the problem is greater in some parts of the world than in others because their climatic conditions are more favourable for mould growth and their synthesis of mycotoxins, these toxins have been associated with diseases of mycotoxicoses in human (Fernandez and Vamond, 1996) and in animals (Richard et al., 1993), they can be hepatotoxic, nephrotoxic, neurotoxic, dermatoxic, genotoxic, hemorrhagic, car-cinogenic or have hormonal effects (Qureski et al., 1990; Cheo, 1997 and Cusmans, 1997). The principle sources of infection are mouldy feed especially hay, barley, various nuts, Soya been and rice which are the main foods frequently contaminated by mold (Jarvis, 1975; Hayes, 1985 and Aziz and Yousef, 1997). The most common mold known to produce mycotoxins were Aspergillus species, Fusarium species and Pencillium species (Cheo, 1997). Molds can produce reproductive failure in animals either as a direct result of established infection in the genital tract or by producing toxic metabolites (mycotoxins) in vitro, which are subsequently ingested and absorbed (Fernandez and Vamond, 1996). The purpose of the present study is to isolate and identify possible toxigenic molds in affected internal organs of bulls. Moreover, to study the direct and indirect effect on reproductive system using mycological and pathological examinations.

MATERIALS AND METHODS

Reproductive organs (testes, epididymis), lung, liver, intestine and kidney specimens of 100 slaughtered bulls were collected from Cairo and Giza abattoirs during the period from April to October 2007. The age of animals ranged between 2 to 3 years. Gross pathological lesions of those specimens were recorded and each specimen was divided into two parts, one was placed in a small sterile box for mycological examination while the other portion was fixed in 10% formol saline solution for histopathological examination.

Mycological examination:-

Prevalence of moulds in tissue specimens
1- Enumeration of moulds was carried out according to A.P.H.A. (1966):
2- Isolation and identification of moulds genus and species were carried out according to Samson
(1979).

3-Extraction of mycotoxins in examined specimens:

Aflatoxins, ochratoxin, zearale-none and penicillic acid were extracted from the examined specimens according to Raybal et al., (1988) and its quantity was determined by HPLC assay using mycotoxin standard curves.

Histopathological examination:-

Fixed tissue specimens of reproductive organs (testes, epididymis), lung, liver, intestine and kidney were prepared by paraffin embedding method, sectioned at 3-5 µm thickness and finally stained with hematoxylin and eosin (Banc-roft and Stevens, 1990).

Statistical analysis:

Data were statistically analyzed using students t-test. One sample t-test according to Snedecor and Cochran, (1980) and by using SPSS statistical program version 9.0.

RESULTS

Mycological results:

Table (1) Analytical results of numbers & percentage of isolated fungal species in relation to total isolated.

<table>
<thead>
<tr>
<th>Isolated fungi</th>
<th>Examined samples (from 100 slaughtered bulls)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Testes(87)</td>
</tr>
<tr>
<td></td>
<td>No.</td>
</tr>
<tr>
<td>A.flavus</td>
<td>163</td>
</tr>
<tr>
<td>A.parasiticus</td>
<td>112</td>
</tr>
<tr>
<td>A.fumigatus</td>
<td>108</td>
</tr>
<tr>
<td>A.aechracus</td>
<td>83</td>
</tr>
<tr>
<td>A.niger</td>
<td>86</td>
</tr>
<tr>
<td>Fusarium spp.</td>
<td>55</td>
</tr>
<tr>
<td>Penicillium spp.</td>
<td>37</td>
</tr>
<tr>
<td>Mucor spp.</td>
<td>44</td>
</tr>
<tr>
<td>Candida spp.</td>
<td>22</td>
</tr>
<tr>
<td>Cladosporium spp.</td>
<td>43</td>
</tr>
<tr>
<td>Total</td>
<td>753</td>
</tr>
</tbody>
</table>
Table (2) Tissue distribution of mycotoxins (types & amounts μg/gm).

<table>
<thead>
<tr>
<th>organs</th>
<th>Aflatoxin</th>
<th>Ochratoxin</th>
<th>Zearalenone</th>
<th>Penicillic acid</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Alpha</td>
<td>Beta</td>
</tr>
<tr>
<td>Testes</td>
<td>19.55</td>
<td>8.32</td>
<td>17.32</td>
<td>11.27</td>
</tr>
<tr>
<td>Epididymis</td>
<td>3.14</td>
<td>3.14</td>
<td>11.44</td>
<td>3.12</td>
</tr>
<tr>
<td>Lungs</td>
<td>22.44</td>
<td>11.22</td>
<td>19.42</td>
<td>5.12</td>
</tr>
<tr>
<td>Liver</td>
<td>18.21</td>
<td>9.43</td>
<td>8.91</td>
<td>2.95</td>
</tr>
<tr>
<td>Intestine</td>
<td>18.95</td>
<td>8.37</td>
<td>9.22</td>
<td>1.22</td>
</tr>
<tr>
<td>Kidney</td>
<td>13.11</td>
<td>7.42</td>
<td>6.14</td>
<td>2.63</td>
</tr>
</tbody>
</table>

Pathological results:
- **Testes** (87 cases) and **Epididymis** (53 cases).

Gross findings:
Reproductive organs, testes and epididymis were of pale color and small size.

Microscopical findings:
The testicular tissue specimens revealed, thickening of the tunics and intense edema of the interstitial tissue with depletion of spermatogoneal cells in seminiferous tubules and intraluminal accumulation of desquamated cells and cells debris. In some areas seminiferous tubules either showed desquamation of the lining cells with the formation of cellular casts with edema and mononuclear cells infiltration of the interstitial tissue (fig.1) or appeared necrotic with complete destruction of sertoli and germinal cells and intraluminal accumulation of cell debris and detritus. Hyalinization of tubular basement membrane and interstitial connective tissue with loss of interstitial cells were also observed in some areas(fig.2). The convoluted tubules of the ductus epididymis were either dilated and cystic and contained varying amounts of cellular exudates rich in neutrophils or were atrophied and compressed by the thickened fibrous intertubular stroma which is heavily infiltrated with lymphocytes(fig. 3). In some areas the tubules were empty and widely separated by dense fibrous connective tissue or were replaced by the proliferative fibrous tissue. Tubular epithelium may become vacuolated. The main fungi isolated was, *Aspergillus spp.*

Lungs (88 cases):
Microscopical examination of the lung tissue specimens revealed 3 types of pneumonia:
A- Hemorrhagic pneumonia (34 cases).
B- Granulomatous pneumonia. (9 cases).
C- Bronchopneumonia (45 cases).

A- Hemorrhagic pneumonia:
Gross findings:
Lung tissue showed severe congestion and multiple large hemorrhagic areas on the pleural surface, which oozed bloody exudates on cut section.

Microscopical findings:
Most of the aspirated blood during slaughtering. Moreover, the alveolar walls appeared to be thickened due to proliferation of septal cells and infiltration of mononuclear inflammatory cells mostly lymphocytes. Some alveolar lumina showed edema fluid and / or fibrin threads together with mononuclear inflammatory cells infiltration mainly lymphocytes (fig. 4). The lining epithelium of the bronchi and bronchioles showed desquamation into the lumen in some lungs. Fungal isolation of this type of pneumonia were mainly Aspergillus spp. and Penicillium spp.

B- Granulomatous pneumonia:
Gross findings:
The lung tissue showed congestion with the presence of minute petechiae. Multiple nodules whitish to grayish in color about 2 mm in diameter were noticed scattered on the external surface of the lung.

Microscopical findings:
Multiple small areas of granulomatous reaction, characterized by central core of lung tissue necrosis and mononuclear cellular infiltration mainly lymphocytes and macrophages which surrounded by thin layer of fibrous connective tissue were observed (fig. 4). Most of the pulmonary tissues revealed fungal hyphae of Aspergillus spp. (fig. 5) together with thickening of the interstitial tissue by mononuclear inflammatory cells (lymphocytes and histocytes) infiltration and alveolar emphysema was also detected in some areas. The most common fungal isolation of this type of pneumonia were Aspergillus spp, Mucor spp. and Fusarium species.

C- Bronchopneumonia:
Gross findings:
Moderate congestion was observed on the surface and serous exudates oozed on cut section.

Microscopical findings:
The bronchial and bronchiolar lumina revealed presence of cellular exudates consisted of lymphocytes and histocytes admixed with exfoliated degenerated and necrotic epithelial cells (fig. 6). Peribronchial and peribronchiolar tissue showed moderate mononuclear inflammatory cells infiltration together with marked proliferation of bronchus associated lymphoid tissue (BALT) were seen in most of
bronchi, in addition, the interalveolar septa showed congested blood capillaries. The main fungi isolated from this type of pneumonia were, *Aspergillus spp*, *Pencillium spp.*, *Mucor sp* and *Candida spp*.

**Liver** (75 cases), **intestine** (42 cases) and **Kidney** (33 cases):  
**Gross findings:**  
The liver appeared congested, yellowish, enlarged and friable. Erosion and necrosis were seen in most of the mucosa and no macroscopically changes were observed in the kidney.  
**Microscopical findings:**  
**Liver:**  
Multiple sporadic necrotized hepatocytes were noticed scattered throughout the hepatic parenchyma with fibroblastic proliferation in the portal areas surrounding bile duct and hepatic artery (fig. 7). The main fungi isolated were *Aspergillus spp* and *Cladosporium spp*.  
**Intestine:**  
Superficial surfaces of the villi showed degeneration of the epithelial cells and coagulative necrosis was observed in some villi. Moreover, the lamina propria was intensely infiltrated with mononuclear inflammatory cells. The main fungi isolated were *Aspergillus spp* and *Cladosporium spp*.  
**Kidney:**  
Renal tubular epithelium revealed degenerative changes and sometime necrotic changes (fig. 8). The main fungi isolated were *Aspergillus spp* and *Mucor spp*.  

**DISCUSSION**  
Mycological results of the present study (tables 1, 2) revealed that the most toxigenic isolated fungi in different organs were *Aspergillus flavus, A. acharacus, Fusarium species* and *Pencillium species* and the toxins produced were aflatoxin, ochratoxin, zearalenone and penicillic acid. Our results of pathology, gave an indication that the reproductive organs (testes and epididymis) were badly affected either directly or indirectly (systemically via inhalation or ingestion) by mycotoxins. Nearly similar findings were noticed by Fenske and Finkgremmels, (1990) and Diekman and Green (1992) who recorded that mycotoxins impair the reproductive efficiency and the mycotoxins of distinct chemical structure act directly or indirectly on testicular tissue presumably by inhibiting early steps of the steroidogenic pathway. Lung specimens of the bulls contained different kinds of fungi (table 1) which had been reported as a common cause of mycotic pneumonia (Carter et al., 1973). In addition, Aspergillus spp. were the most predominant fungi isolated as recorded by Cordes and Shortidge (1968) and
Ainsworth and Aaustwich (1973). *Aspergillus spp.* is considered to be an opportunistic infectious organism and occurred by inhalation of fungal spores originating from mouldy hay or soil (*Pusterla et al.,* 1996) or by ingestion of contaminated silage or hay feed to animals (*Jones et al.,* 1997). Generally, *Aspergillus spp.* is capable of causing diseases by several ways, including direct tissue invasion, colonization, and a reaction involving the immune system (*Mazen et al.,* 1984) depending largely on local or general immunological state of the host (*Quinn et al.,* 1994). The presence of toxigenic fungi in pneumatic lungs provides a potential for pneumatic problems which come in accordance with *Jarvis (1975).* Regarding pathological results, histopathological findings of the bull lungs revealed that, there were 3 types of pneumonia (hemorrhagic *p. granulomatous* and *P. broncho-pneumonia*) and the most prevalent fungal isolation of them was *Aspergillus spp.* These results come in harmony with *Bendary, (1986); Fatma et al. (2001)* and *Amer et al.,* (2007) they stated that, *Aspergillus spp.* was the main cause of mycotic pneumonia in camels. Granulomatous pneumonia histologically exhibited typical *Aspergillus* granulomata which were previously encountered in bovine lungs by *Srinivas et al. (1984); Maity and Deb (1993)* and *Pusterla et al. (1996).* *Aspergillus spp.* was recovered from cases of hemorrhagic pneumonia of slaughtered bulls either alone or mixed with other types fungi. These results coincide with the findings of *Maity and Deb, (1993)* and *Fatma et al. (2001)* who observed that, hemorrhagic pneumonia developed in some cases of *Aspergillus* infection. Moreover, *Aspergillus spp.* plays a great role in the development of inflammatory alterations observed in the bronchopneumonic lungs of our results as reported by *Rabab et al., (2003).* In addition, *Kathleen and Arther (1996)* recorded that, mixed *Aspergillus* infection with other types of fungi such as *Penicillium spp.* and *Fusarium spp.* induce its pathogenicity by the release of several factors including proteolytic and hydrolytic enzymes and inflammatory stimulants. *Gobrial et al. (1991)* stated that, the fungal infection may be concomitant with other types of infections. Bacterial infection impairs the host defenses and lowers the resistance of the tissue and predispose to fungal infection. Pathological alterations in our study in the liver showed multiple sporadic necrotized hepatocytes throughout the hepatic parenchyma, this result was supported by *Cheo (1991)* and *Amer et al. (2007)* who stated that, mycotoxins causes hepatotoxicity due to hepatocellular injury leading to an increase in the permeability of
liver cell membrane. The lesions observed in the intestine of the bulls come in agreement with those of Chihaya et al. (1992) and Jensen et al. (1994) who found coagulative necrosis in the fungal infected intestine. The destructive effect of kidney mainly degenerative and necrobiotic changes in most of the renal tubular epithelium which comes in agreement with those of Kuiper and Goodman (1990) and Diekman and Green, (1992) who suggested that, mycotoxins induce renal failure. Moreover, Plestina et al. (1990) found that ochratoxin is nephrotoxic.

Conclusion:
From the above mentioned results, we found that the reproductive system of bulls was badly affected by fungal infection directly and indirectly with affection of the other organs of the body. Preventing mould growth is the best method to stop production and to control the economic losses mainly the reproductive performance.

REFERENCES
Chihaya, Y.; Matsukawa, K.;


Fig. (1) Testis of a bull showing desquamation of the cellular elements of seminiferous tubules, oedema and mononuclear cell infiltration of the interstitial tissue (H&E, X100).

Fig. (2) Testis of a bull showing tubular necrosis and hyalinization of tubular basement membrane and interstitial C.T (H&E, X400).
Fig. (3) Epididymus of a bull showing fibrous thickening of the intertubular stroma and atrophy of the tubules (H&E, X100).

Fig. (4): Lung of a bull showing hemorrhagic inflammation (multiple and extensive areas of hemorrhage in alveolar lumina and in the interstitial
Fig. (5): Lung of a bull suffered from granulomatous pneumonia showing fungal hyphae of Aspergillus spp. (arrows) (H&E; X100).

Fig. (6): Lung of bulls showing exudates admixed with exfoliated necrotic epithelium in the bronchial lumina (H&E, X100).
Fig. (7): Liver of bulls showing fibroblastic proliferation in the portal area surrounding bile duct and hepatic artery (H & E, X100).

Fig. (8): Kidney of bulls showing necrobiotic changes in the renal tubules
دراسات هستوپاتولوجية على الأثر المباشر وغير المباشر للعدوى الفطرية على الجهاز التناسلي للطواطق
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معهد بحوث التناسليات الحيوانية

المتخصّص العربي
أجرت هذه الدراسة على عدد 100 من الطناّقات مذبوحة في مجازر القاهرة والجيزة وقد تمّ أخذ عينات نسيجية من الخصية والرثة والكبد والأمعاء والكلى وقد تم عزل الفطرية المختلفة من تلك الأنسجة وتتم استخلاص السموم من الفطريات المعزولة وكذلك أجريت فحص باثولوجي لهذه الأنسجة وكان أكثر الفطريات المعزولة سمية هو فطر الأسبريلجس فلابس والاسيرجس أكراس والفيراومة والبيسكس حيث كانت السموم المستخلصة منهم هي الإفلاكوسين، الأوكتراتوكسين، الزيرالينون وحمض البيسيليك.

وقد أوضح الفحص الهستوپاتولوجي للنساء (27) والبرخ (35) وقّد أن هناك بؤر عديدة لتركنات اضطرابات في أنابيب الخصية وأخرى تناقص طبقات الخلايا المبطنة لجدار أنابيب الخصية مع وجود تركنات في البرخ من حيث تتمّت بالإضافة إلى وجود إرباكس وخلايا وحيدة الأنسوية في النسيج البيني.

أظهرت الدراسة وجود 3 أنواع من الالتهابات الرثية: الالتهاب الرثي المصاحبة بورم نسيجي (9 حالة) - الالتهاب الرثي الشعبي (95 حالة) - وفي الكبد (70 حالة) وجد خلايا تكرازية وتفاوت في المنطقة المحيطة بالقناة المرارية والشركير الكبدى.

الفحص الهستوپاتولوجي للأمعاء (17 حالة) أظهرت وجود ارتفاعات غليظة لخلايا التهابية وحيدة الأنسوية في طبقات جدار المعدة أما في الكلي (33 حالة) شوهد تركنات تنكرازية في معظم الخلايا المطلانية لانابيب الكلي.

وقد تم رصد ومناقشة نتائج الدراسة الفطرية وثرت ذلك على النتائج الباثولوجية في أجهزة الجسم المختلفة مثل الرثة والكبد والأمعاء مما يؤثر تأثير مباشر أو غير مباشر على الجهاز التناسلي للطناّقات وكفاءته التناسلية ودرجة الخصوبة.

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