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Detection of Pathogens of Condemned Lungs of One Humped Camels (*Camelus dromedarius*) Slaughtered in Matrouh Abattoirs, Egypt

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Abstract: This study was carried out on 175 camels with the aim of identifying bacterial, parasitic and fungal species involved in lung lesions of camel's slaughtered at Matrouh main abattoirs. All slaughtered camels were originated from northwestern coast. A total of 175 lungs were inspected during the study, of which 50 (28.6%) possessed pneumonic lesions. Bacterial and mycotic growth was observed from 45 (25.7%) of the pneumonic lung samples. A total of 70 bacterial and mycotic species were isolated and identified. These included *staphylococcus aureus* (28.6%), *Bacillus* species (21.4%), *Klebsiella species* (10%) *Escherichia coli* (8.6%), *Corynebacterium pyogenes* (5.7%), *Streptococcus pyogenes* (2.9%), *Streptococcus pneumoniae* (1.8%), *Pasteurella multocida (*2.9%), *Manhemia haemolytica (*1.4%), *Actinomyces pyogenes* (1.4%), *Aspergillus fumigatus* (5.7%), *Aspergillus flavus* (1.4%) and *Candida albicans* (1.4%). Serological detection of *Aspergillus fumigatus* antibodies using IHA was found to be (14%). Hydatidosis was found in a rate of (8%). Some histopathological changes were detected in pneumonic lung tissues. In conclusion, proper abattoir records can serve as indicator for filed disease condition and consequently used by the relevant authority in planning prevention/control programmes.

Key words: Camels · Abattoir · Lung · Microbial · Parasitic

INTRODUCTION

The one-humped camel is widely distributed in the Horne of Africa, North African countries, the Arab peninsula and some countries of Asia. Bedouin use them as a means of transportation in the desert; they can also be used for tourism or reared for production of meat, milk, hair or hide [1].

Respiratory tract infections are of common occurrence in various species of domestic and farm animals [2]. Virus, bacteria, fungi and parasites have been incriminated as the main causative agents of pneumonia in mammals [3]. These agents may represents risk to camels, other livestock and even human population [4]. Camels are uniquely adapted to hot and arid environment and seem to be spared from devastating epidemic infection that threaten other animal species sharing the same habitat, there are however a number of economically important diseases that affect camels [5, 6].

Pulmonary diseases are among the emerging problems of camels that are causing considerable loss in production and death [7, 8]. Yet there is need to identify the causes of respiratory diseases in camels in order to design better control strategies. The objective of this present work was to carry out some bacteriological, mycological and parasitic causes as well as some histopathological findings of pneumonic lung in one humped camels (Camelus dromedarius) slaughtered in Matrouh Abattoirs.

MATERIALS AND METHODS

Study Animals and Area: The study was conducted on one hundred and seventy five one-humped camels (6 months to 9 years old) presented for slaughter at Matrouh main abattoirs. All camels slaughtered were originated from northwestern coast approximately 500 km west of Cairo where camel rearing is common.

Data Collection and Sampling Procedure: Although records were not available concerning the pervious health status, camels were found to be apparently healthy at the ante-mortem examination. Postmortem examination (175 lungs) was made by routine visual, palpation and

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incises methods of the airways, lungs and the associated bronchial lymph nodes for the presence of any lesion [9]. Tissues from apparently affected lungs were collected and divided into two portions. One portion was fixed in 10% neutral buffered formalin for histopathological examination. The other portion was placed in sterile plastic bags, kept in an ice box and subjected to bacterial, fungal and parasitological examinations. Blood samples were taken from examined animals for serodiagnosis of aspergillosis in pneumonic camels.

Methods

Bacteriological Isolation: A full loop was taken from the infected lungs and inoculated into peptone water and incubated at 37°C for 24 hrs. The inoculated broth was cultivated onto nutrient agar, Selenit-F broth, Mac-Conkey agar, blood agar and S.S agar plates. The inoculated plates were then incubated at 37°C for 18- 24 hrs.

Mycological Isolation: The samples were cultured onto duplicate sets of Sabouraud's dextrose agar plates containing antibiotics, one plate was incubated at room temperature (22-25°C) and the other was incubated at 37°C. The plate showing no growth was discarded after 5 days incubation.

The obtained bacterial and fungal isolates were identified using standard biochemical scheme [10-15].

Sensitivity Test: The antimicrobial sensitivity patterns of the bacterial isolates were done in vitro against the following chemotherapeutic agents, Spectrama 10µg, Streptomycin 10µg, Ampicillin 10µg, Gentamicin 30µg, Tetracycline 30µg, Cephaloridine 30µg, Erythromycin 10µg [16]. Fungal isolates were tested in vitro against Ketoconazole, amphotricine B and Fluconazole.

Indirect Haemagglutination Test (IHA): Indirect haemagglutination test (IHA) for serodiagnosis of Aspergillosis in pneumonic camels was carried out by a kit supplied from Fumoze-diagnostics, France according to Senet and Brisset [17].

Histopathological Examination: Tissue samples were collected and partially fixed in 10% neutral buffer formalin. The fixed specimens were processed and imbedded in paraffin wax, section at 3-5 micron thickness were prepared and stained with H&E stain for histopathological examination [18].

RESULTS

Out of 175 lungs examined, 50 (28.6%) were pneumonic. Forty five samples (25.7%) were positive for bacterial and mycotic isolation while 5 (2.8%) samples were found to be negative for bacteriological and mycological isolation. The incidence of different bacteriological and mycological isolates are shown in Table (1). *Staph. aureus* was the most frequently isolated bacteria.

Table 1: The incidence of different bacterial and mycotic isolates recovered from pneumonic lungs of slaughtered camels

Isolated bacteria	Number	Percent (%)
Staphylococcus aureus	20	28.6
Bacillus species	15	21.4
Klebsiella species	7	10.0
Streptococcus pneumonia	6	8.6
Escherichia coli	6	8.6
Corynebacterium pyogenes	4	5.7
Streptococcus pyogenes	2	2.9
Pasteurella multocida	2	2.9
Manhemia haemolytica	1	1.4
Actinomyces pyogenes	1	1.4
Aspergillus fumigatus	4	5.7
Aspergillus flavus	1	1.4
Candida albicans	1	1.4
Total isolates	70	100.0

*Total samples =50. *Total bacterial positive samples =45

*The percentage was calculated according to the number of total isolates

Table 2: Types of mixed bacterial and mycotic isolates of pneumonic lung of slaughtered camels.

Type of isolated microorganisms	Number	Percent (%)
Staphylococcus aureus + *Escherichia coli*4	4	8.8
Staphylococcus aureus + *Corynebacterium pyogenes*	3	6.6
Staphylococcus aureus + *Klebsiella species*	3	6.6
Staphylococcus aureus + *Streptococcus pyogenes* + *Candida albicans*	1	2.2
Staphylococcus aureus + *Pasteurella multocida* + *Aspergillus flavus*	1	2.2
Staphylococcus aureus + *Bacillus species* + *Aspergillus fumigatus*	6	13.3
Staphylococcus aureus + *Actinomyces pyogenes* + *Aspergillus fumigatus*	1	2.2
Total isolated bacterial isolates	19	42.2

The percentage was calculated according to the number of positive samples (45)

Table 3: The results of the antibiotic sensitivity test of isolated bacteria from pneumonic lungs of slaughtered camels

	Antibiotic discs						
Isolated bacteria	Spectrama	Gentamycin	Streptomycin	Cephaloridine	Erythromycin	Tetracycline	Ampicillin
Staph. aureus	+++	+++	+	+++	++	+	++
Strept. pneumonia	+++	+++	-	++	+	_	++
Escherichia coli	+++	+++	-	+++	+	++	+
Corynebacterium pyogenes	+	++	+	-	+	+	+++
Streptococcus pyogenes	+++	+++	+	+++	++	++	++
Pasteurella multocida	+++	+++	-	+++	+	+	-
Manhemia haemolytic	+++	+++	-	+++	+	+	-
Actinomyces pyogenes	+	++	+	+	+	+	++

+++ highly sensitive. ++ moderately sensitive. + Low sensitive. - nonsensitive

Table 4: Antimycotic sensitivity pattern of mycotic isolates

	Antimycotic discs		
Mycotic isolates	Amphotrecine B	Fluconazole	Ketoconzole
Aspergillus fumigatus	+++	++	+
A. flavus	+++	++	+
Candida albicans	+++	+	++

Table 5: Antibody response to Aspergillus fumigatus using IHA test

	Different titres of IHA							Positive culture		Positive IHA	
No. of animals	□1/80	1/80	1/160	1/320	1/640	1/1280	1/2560	No.	%	No.	%
50	33	6	4	3	*2	*1	*1	4	8	7	14

* Positive culture. IHA- Indirect haemagglutination - Positive results more than or equal1/320

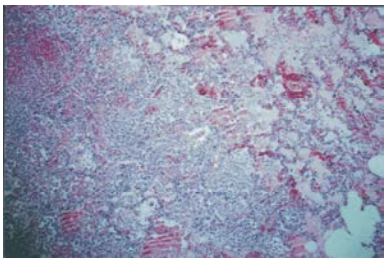

Fig. 1: Interstitial pneumonia of lung of camels (H&E 100 X)

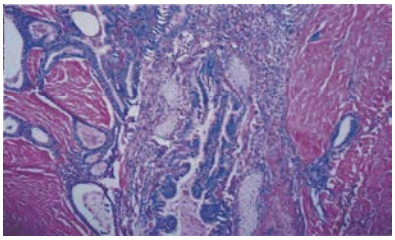

Fig. 2: Bronchitis, bronchiolitis with lung fibrosis of camels (H&E 100 X).

In this study, *Asperillus fumigatus, Asperillus flavus* and *Candida albicans* were found in 5.7%, 1.4% and 1.4% of pneumonic camel's lungs respectively, Tables (1). The isolation rate of mixed bacterial and mycotic species and their involvement are shown in Table (2).

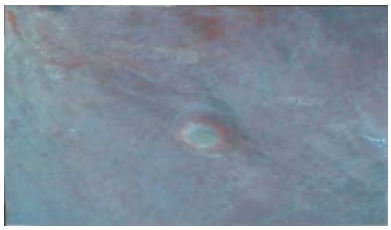

Fig. 3a: Hydatid cyst in lung of camels

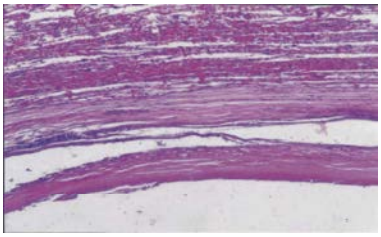

Fig. 3b: C. S. Parasitic cyst of hydatid cast in lung tissues of camels (H&E 100 X)

The antibiotic sensitivity patterns of the bacterial and mycotic isolates were illustrated in Tables (3 and 4) respectively.

The results of serological detection of *Asperillus fumigatus* antibodies illustrated in Table (5). Serum samples with titre equal 320 or more were considered to be positive.

The histopathological examination, showed interstitial pneumonia Fig. (1), bronchitis, bronchiolitis with lung fibrosis of camels Fig. (2). Parasitic cyst was found in 4 (8%) examined lungs. The wall of the cyst consisted of more or less a cellular homogenous laminated cuticular structure investing a layer of epithelial cells, Figs. (3 a & b).

DISCUSSION

Slaughter houses provide an excellent opportunity for detecting diseases of both economic and public health importance [19].

In the present study, a fair number of bacterial and mycotic isolates (70) were collected from 45 pneumonic lungs of which 19 (42.2%) revealed more than one bacterial and mycotic species. Macroscopic examination of lung samples revealed that 50 samples (28.6%) were pneumonic. This rate is considerably higher than that recorded by Al-Tarazi *et al* [1], Al-Rawashdeh *et al.* [20] and Mahmoud *et al.* [21], where the infection rates of the lungs examined were 10%, 12% and 10.2% respectively. On the other hand, Abu-Bakr *et al*. [22] and Awol *et al.* [23] recorded an infection rates 77.5% and 68.1% respectively. The variation in rates could be due to variation in sample size or due to variation among geographical areas.

In the present study, failure of bacterial isolation in 5 (2.8%) lung tissue with observable pneumonia even though there was evidence long standing inflammatory response. This may be suggestive of mycobacterial infection and in addition probable viral implication or other anaerobic bacteria [6].

Identification of the pneumonic pathogens in the present work cleared that *staph. aureus,* was the most common pneumonic bacteria isolated from lung tissue at a rate of 28.6%. This result was higher than that recorded by Al-Tarazi *et al.* [1] as 4%, Mahmoud *et al.* [24] as18% and Awol *et al.* [23]as 21.1% and almost in agreement of the result recorded by El-Tigani *et al*. [25]. The isolation rate of *Bacillus* spp. and *Klebsiella* spp. were 21.4 and 10% respectively. This in accordance with the previous results of Zubair *et al*. [7] and Kane *et al*. [8] who reported that, *Bacillus* spp. and *Klebsiella* spp. were commonly isolated from pneumonic lesions in camels. Lower rates were recovered by Al-Tarazi *et al.* [1] as14.66 and 5.33%, Aziziollah *et al*. [26] as 16.6 and 1.27% and Abu-bakr *et al*. [22] as 5 and 8.1% for *Bacillus* and *Klebsiella spp.* respectively. In addition *Klebsiella spp.* was less prominent and might be considered as secondary invader.

Strept. pyogenes and *Strept. pneumoniae* were recovered at a rate of 2.9 and 8.6%, respectively. This rate is considerably lower than that recorded by Awol *et al.* [23] and El-tigani *et al.* [25] who recovered *Streptococcus* species at rate of 19.3% and13.9% respectively. In addition, Mahmoud *et al*. [24,] and Osman *et al*. [27] reported the isolation of *Sterpt. pyogenes* and *Sterpt. pneumoniae* from pneumonic lesions of camel lung's.

Escherichia coli was isolated at a rate of 8.6% in this study. This is much lower than that of Al-Tarazi *et al*. [1] and Awol *et al.* [23], who recovered *E. coli* at a rate of 26.7 and 17.5%, respectively. But, higher than 3 and 6.2% that reported by Zubair *et al*. [7] and Al-Doughaym *et al*. [28] respectively. However, the results coincide with that of Mahmoud *et al*. [24] who reported an isolation rate 11.1% in camels with pneumonia. The isolation rate of *E. coli* correlates with the natural habitat of *E. coli*, where it can survive in faecal particles, dust and water for weeks and months [29].

Corynebacterium pyogenes was isolated in a percentage of 5.7% of pneumonic lesions. This was in agreement with previous results of Kane *et al*. [8] who reported that, this pathogen was involved in pneumonia of camels under condition of stress, poor sanitation and immunosuppression.

In this study, *Pasteurella multocida* and *Mannheimia hemolytica* were isolated at a rate of 2.9 and 1.4%, respectively. In contrast, Al-Tarazi *et al* [1] and Mahoud *et al*. [24] recoded higher rates of *M. hemolytica* and *P. multocida* 6.6 and 7.4% respectively. *Pasteurella* species are commensals residing in the nasopharyngeal microflora and are all capable of causing infection when the body defense mechanism are impaired also Quinn *et al*. [30] and Radostitis *et al*. [31] reported that *Mannheimia hemolytica* was recognized as a cause of primary and secondary pneumonia and a number of non-specific inflammatory lesions in various species of domestic animals. However, it was isolated from the deep pulmonary tissues of the examined cases. This may be an indication of the presence of suitable conditions in the lungs facilitating the invasion of pasteurlosis into deep tissues.

The percent of *Actinomyces pyogenes* isolates in the present study was 1.4%. Al-Tarazi *et al.* [1] isolates *A. pyogenes* from abscesses of lung of camels at a rate of 6.6%.

It is commonly to detect pulmonary mixed infection since the bovine respiratory air passages act as reservoirs for potential pathogenic microorganisms, which develop pneumonia on the onset under stress factors, poor sanitation and climatic conditions [32].

In this study, pneumonic mixed pathogens are mainly *staph. aureus* and other organisms, Table 2 whish demonstrate the complexity of the disease where *staph. aureus* may predispose the animals to infection by other pathogens these results were in agreement with that of Taha *et al.* [33] and Sayed and Zaitoun [34].

Concerning mycotic pneumonia, *Aspergillus fumigatus, Aspergillus flavus* and *Candida albicans* were isolated from 5.7, 1.4 and 1.4% of pneumonic lungs, respectively. The isolated fungi were mixed with bacteria. These results are in agreement with that of Mahmoud *et al*. [24] and Mahmoud *et al*. [35].

It is known that the camel is one of common intermediate hosts of *Ecchinococcus granulosus* and hydatid cysts have been reported in camels from almost all countries [36]. In Egypt, the prevalence was 4.3 to 8.2% [33]. In the present study, the prevalence of hydatid cysts was 8% in collected pneumonic lungs. This rate was in parallel with that of Dyab *et al*. [37] as7.67%, Mahmoud *et al*. [24] as 7.5% and Azlaf and Dakakk [38] as 12.03%. Nouh and Mohamed [39] recorded an infection rate 29.11% at abattoir study in Sharkia. The prevalence varies from one camel population to another as well as from place to place. Sakamoto and Guitierrez [40] mentioned the close relationship between ecchinococcosis and pneumonia.

Concerning the detection of *A. fumigatus* antibodies, 14% of examined serum samples of pneumonic camels were positive by IHA. Serological tests are important in the diagnosis of systemic mycosis since the results often permit differentiation between colonization by fungi and a true mycotic infection [41]. El-Naggar *er al*. [42] recorded nearly the same result in camels in south-eastern Egypt. However, Kazuo *et al*. [43] and Sweeney *et al*. [44] concluded that prolonged use of antibiotics and/or corticosteroids therapy represent a possible predisposing factors for clinical mycotic infection.

In the present work, some histopathological examination revealed interstitial pneumonia was characterized by mononuclear cell infiltration mainly lymphocytes and macrophages Fig. (1). bronchitis and bronchiolitis were found in some pneumonic lungs showing hyperplasia of the bronchial lining epithelium, prebronchial lymphocytic aggregation and fibrosis of bronchial lumen leading to bronchiolar obliteration Fig. (2). The same findings were recorded by Taha *et al*. [33]. Also the lung showed cross section of hydatid cyst characterized by fibrous lamellar wall and centrally contained gelatinous materials Fig. (3 a and b). these results were in agreement with that of AL-Sadi *et al*. [45].

The sensitivity of the different bacterial isolates which represent the main causative agents is shown in Table (3). It is found that most of the bacterial isolates were sensitive to Spectrama, Gentamycin, Cephaloridine, moderate sensitive to Ampicillin, Erythromycin and resistant to streptomycin, while mycotic isolates Table (4) were highly sensitive to amphotrecine B. These results coincide with that of Mahmoud *et al*. [24], Seddek [46] and Robert *et al*. [47].

CONCLUSION

The findings of this present study suggest that meat inspection practices require some improvement. The hostile attitude of the butchers and animal traders has resulted in the lenient nature of meat inspection. In conclusion, proper abattoir records can serve as indicators for field disease conditions and, consequently, an aid in assessing the health status of animals and consequently used by the relevant authority in planning prevention/control programmes.

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