Some studies on tuberculosis in camel

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SUMMARY

A total of 704 camels at different abattoirs were investigated in this study by different techniques for diagnosis of tuberculosis (T.B.). These examined camels were divided into two groups, the first group with 569 camels which were kept in close contact with cattle and the second group with 135 camels were kept in close farm. Both groups were examined for the presence of tubercles like lesions in different organs. In 1st group, tubercles granuloma could be detected in 25 camels mainly in lungs, lymph nodes and spleen. Eight camels of these group were reported as ELISA positive. By bacteriological examination of them, *M. bovis* could be isolated from 5 camels as well as typical histopathological lesions were observed which formed mainly of granulomatous reactions at different stages and milliary T. B. in 5 camels. On the other hand, in 2nd group 4 camels was positive (+ve) for granuloma formation and one positive (+ve) for ELISA while were negative for bacteriological and histopathological examination for T.B.

We concluded that the incidence of camels infected with T.B. in Egypt is 0.7% and this incidence present among camels kept in close contact with cattle.

INTRODUCTION

Camels play important socio-economic roles within the pastoral and agricultural systems in the arid and semiarid zones of Asia and Africa. The survival of millions of human being is dependent on the camel in such areas, for meat, milk, hair production and still as an important mean of draught and transport for large sector of pastoral societies.

Tuberculosis is a disease that had already been diagnosed around the turn of the century in dromedaries in Egypt (Wernery and Kaaden, 2002).
In tropical developing countries where tuberculosis has received little attention, substantial economic losses can occur especially in cattle. Tuberculosis as a zoonosis, also plays an important role among nomadic people where milk and milk products are consumed raw (Seifert, 1992). This also true for camel milk (Donchenko et al., 1975b) isolated *M. bovis* strains from 46 pooled milk samples from 712 lactating camel cows in Russia. Tuberculin tests were performed in these herds whereby 9.1% were reactive. Other than unheated camel milk, circus and zoo camels with active disease also present a danger to man (Dekker and Van Der Schaaf, 1962).

Tuberculosis persists as a costly zoonotic disease in numerous countries despite extensive eradication and control efforts, tuberculosis in humans may result from exposure to any one of the tubercle bacilli included within the mycobacterium tuberculosis complex (i.e. *M. tuberculosis, M. bovis, M. africanum, M. pinnipedii* and *M. microti*). *Mycobacterium bovis*, unlike *M. tuberculosis* has a wide host range in the species most often isolated from tuberculous cattle and has several wild life maintenance hosts (Waters et al., 2006).

Duguid et al. (1983) reported that the tubercle bacillus doesn't produce any toxin apart from the hyper sensitivity reaction which develops only in animals previously infected with organism. The bacilli ingested by macrophages multiply slowly ingeneration time 6 to 12 h. depending on their being able to resist destruction by lysosomal enzymes. Macrophages died and disintegrated after laden with numerous bacilli and liberated bacilli continue to multiply extracellularly in tissue fluid or after ingestion by other phagocytes. Termination of infection depended on the development of cell mediated immunity and a consequent production of activated macrophages which have increased ability to kill ingested bacilli.

Clinically tuberculosis divided into primary infection (Ghonfocus) which develops at the site of implantation of single air borne particle bearing one or few bacilli which mainly occur in lungs or some times occur in intestine as a result of ingestion of food contaminated with *M. bovis*. From the primary lesion the bacilli are carried by lymphatic drainage to the regional lymph nodes which may be progressive enlarged and followed by caseation and calcification. Haematogenous spread occurs with implantation of bacilli in many organs leading to millary tuberculosis. The post primary tuberculosis developed in previously
infected animal either as a result of endogenous reactivation of latent disease or exogenous reinfection in which one or more lung lesions progress to caseation and cavitation and involving the bronchial tree create a case of open tuberculosis (Crofton et al., 1992).

The diagnosis of camelid tuberculosis in living animals faces many difficulties. None of the tests available can diagnose tuberculosis with certainty. Intradermal tuberculin test, which is the classical diagnostic test often gives nonspecific reactions in camelids (Paling et al., 1988).

The aim of the present study was to determine the incidence of T.B. infection in camels as well as to compare between the bacteriological and histopathological techniques used for diagnosis of T.B. in camels.

**MATERIAL AND METHODS**

* Examined animals:

This study performed on 704 camels at abattoirs. These camels were classified according to place which come from into 2 groups:

1*st* group: 569 camels belonged to farmers who kept them in close contact with cattle and other animal species from different areas in Egypt.

2*nd* group: 135 camels which kept in closed farms from different localities in Egypt.

* Postmortem examination and samples collection:

Tissues with high infection expectation were collected from each examined animal both males and females, included lungs, spleen, the anterior and posterior mediastinal lymph nodes, left and right bronchial and left and right medial retropharyngeal lymph nodes (Table 1-A).

Each tissue specimen were divided into two parts, one part placed in sterile containers and submitted to the laboratory for bacteriological examination. The second part of these organs were fixed in 10% formalin and subjected to pathological examination. Other tissues with a low expectation of infection (Table 1-B) were sliced in-situ and examined visually. When lesion resembling tuberculosis were found they were submitted for bacteriological examination and histopathological examination up to a maximum of 3 lesions were examined from each animal (Corner et al., 1990). Serum samples for ELISA were collected from suspected animals.
Table (1): Tissues examined for lesions of tuberculosis in animals.

<table>
<thead>
<tr>
<th>A. Tissues collected for bacteriological examination</th>
<th>B. Tissues examined incision at the time of slaughter</th>
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</thead>
<tbody>
<tr>
<td>Lung and spleen</td>
<td>* Mandibular lymph node</td>
</tr>
<tr>
<td></td>
<td>* Parotid lymph node</td>
</tr>
<tr>
<td></td>
<td>* Lateral retropharyngeal lymph node</td>
</tr>
<tr>
<td></td>
<td>* Tonsils</td>
</tr>
<tr>
<td>Head</td>
<td></td>
</tr>
<tr>
<td>Medial retropharyngeal lymph nodes&lt;sup&gt;1&lt;/sup&gt;</td>
<td>Thorax</td>
</tr>
<tr>
<td></td>
<td>* Tracheobronchial lymph nodes</td>
</tr>
<tr>
<td></td>
<td>* Lungs</td>
</tr>
<tr>
<td>Mediastinal lymph nodes</td>
<td>Abdomen</td>
</tr>
<tr>
<td></td>
<td>* liver and hepatic lymph node</td>
</tr>
<tr>
<td></td>
<td>* spleen</td>
</tr>
<tr>
<td></td>
<td>* Kidneys</td>
</tr>
<tr>
<td></td>
<td>* mesenteric lymph nodes</td>
</tr>
<tr>
<td>Anterior and posterior tracheobronchial lymph nodes</td>
<td>Carcasses</td>
</tr>
<tr>
<td></td>
<td>* Caudal cervical lymph nodes</td>
</tr>
<tr>
<td></td>
<td>* Subiliac lymph nodes</td>
</tr>
<tr>
<td></td>
<td>* Medial iliac lymph nodes</td>
</tr>
<tr>
<td></td>
<td>* Supramammary lymph nodes</td>
</tr>
<tr>
<td></td>
<td>* Politeal lymph nodes</td>
</tr>
<tr>
<td></td>
<td>* Internal iliac lymph nodes</td>
</tr>
<tr>
<td></td>
<td>* Gluteal lymph nodes</td>
</tr>
<tr>
<td></td>
<td>* Lateral iliac lymph nodes</td>
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</tbody>
</table>

<sup>1</sup> Includes left and right lymph nodes.

*Direct smears:*  
Direct smears were made from lesions for staining with Ziehl – Neelsen stain (Dwight and Yuan, 1999).

*Bacteriological examination of collected specimens (Marks, 1972):*  
All tissues submitted to the laboratory were examined bacteriologically for Mycobacterium species. The isolation of typical Mycobacterium species was used as the definitive test for the diagnosis of camel tuberculosis, whether from animal with suspected tuberculous lesion or from tissues without visible lesions.

The collected tissue samples were dissected from fat and connective tissues and 2 gram portion of tissues from each specimen was
gross lesions (tuberculuous like lesion in necropsid animal) could be detected in 25 camels only. By bacteriological examination of them. *M. bovis* could be isolated from 5 camels as well as typical histopathological lesion for T.B. was detected and 8 from this group of camels were recorded as ELISA positive. The impression smear of infected lung which stained by Ziehl Neleelsen showed the acid fast bacilli of T.B. (Fig. 1).

Regarding to 2nd group of camels (kept in closed farm) only four camels were positive for P/M examination and one camel was positive for ELISA while all camels gave negative results for bacteriological and histopathological examination for T.B.

Different organs of slaughtered camels were examined as shown in table (1). The post mortem changes of examined organs mostly showed numerous milliary tubercles of the same widely scattered on the surface and deep in the lung parenchyma. In addition to large areas of the lung tissue appeared solid and caseated. Enlargement of bronchomediastinal lymph nodes with the presence of white nodules 1 mm to 2 cm in diameter and prominent caseous spheroidal masses in the spleen. In sectioning a tubercle a gritty sensation and grating sound indicate the presence of calcareous material. Histopathological examination of the lung revealed tuberculuous pneumonia with large area caseated and necrosed alveoli with thickening of the pulmonary blood vessels and congested alveolar capillaries (Fig. 2) with severe hyperplasia of the bronchii (Fig. 3), thickening of the pleura with productive and proliferative response of fibrous tissues (Fig. 4). Typical characteristics of tuberculuous lesions with caseous material in the central surrounded by aggregation of epithelioid cells and large number of lymphocytes with the tendency for fibrous tissues proliferation (Fig. 5), the caseated center undergo calcification with intense lymphocytic cell infiltration, fibrous connective tissues reaction and scanty langrhans giant cells were seen in the marginal zone of old tubercles (Fig. 6). Old lesions of the lung where most of the lung parenchyma appeared fibrosed and bronchi surrounded with fibrous connective tissues with scanty lymphocytic infiltration (Fig. 7). Sometimes lung appeared with different myriad tubercles 2-3 mm in diameter all of the same age and size named military tuberculosis with dilated alveoli and fibrosis of its wall with some necrotic and disintegrate cells in its lumen (Fig. 8).

Lymph nodes appeared with severe haemorrhage (Fig. 9) with the development of the typical features of granuloma began with
homogenized with 2 ml of sterile distilled water in a sterile mortar containing sterile sand. Two ml of 4% H$_2$SO$_4$ were added to the mixture and incubated at 37 °C for 30 minutes. The mixture was diluted with 16 ml of sterile distilled water and centrifuged at 3000 rpm for 20 minutes. The supernatant fluid was poured off onto disinfected and the obtained sediment was inoculated into four lowensteinjensen slants (BioMerieux), then incubated at 37 °C for 8 weeks. The inoculated slants were examined daily over a week. Suspected colonies were subcultured and identified according to Chadwick (1981).

**Enzyme Linked Immunoabsorbent Assay (ELISA) technique:**

ELISA for serological diagnosis of tuberculosis in camels was performed as described by Rilacco *et al.* (1990). Microtitre plates were coated with bovine 50 µl PPD diluted in (10 µg/ml) carbonate bicarbonate buffer, (pH 9.6) and incubated for 20 h. at 4°C in humified atmosphere. After washing with PBS tween 20 (0.05% w/v) well were blacked with dried milk diluted in PBST (10% w/v). The blocking buffer tipped off and 100 µl/well of tested serum samples diluted 1:50 were added and incubated at 37 °C for 60 minutes. After 3 washing with PBS (pH 7.4) containing 0.01% tween 20, 150 µl of protein A horseradish peroxidase conjugate (1:1000) was added to each well and then incubated at 37 °C for 60 minutes. After washing again working solution of ABTS substrate (100 µl/well) was added and incubate at 37 °C for 15 minutes.

The optical density recorded at 405 nm in Dynatech microELISA reader. An ELISA reading that is equal to/or higher than double fold of the ELISA reading of negative control is considered positive.

**Histopathological examination:**

Post-mortem examination was carried out on the slaughtered camels. Tissues specimens were taken from the inspected tubercules and fixed in 10% neutral formalin. Processed routinely for embedding in paraffin and sectioned at 4-5 µ thick. Sections were stained with haematoxylin and eosin and examined microscopically and for the detection of acid fast bacilli in tissues, sections were stained with Ziehl-Neelsen stain according to (Bancroft *et al*., 1996).

**RESULTS**

The results of different techniques for diagnosis of tuberculosis in examined camel groups are summarized in table (2).

From 569 camels which kept in contact with cattle (1st group),
clusters of neutrophils surrounded the invading bacilli (Fig. 10). This was replaced by whorl of lymphocytes and macrophages that takes on a different appearance where the cells became reminiscent of epithelial cells with abundant eosinophilic cytoplasm that termed epithelioid cells (Fig. 11, 12) calcified tuberclus was also observed with aggregation of epithelioid cells and lymphocytes (Fig. 13).

Spleen appeared with thickening of the splenic blood vessels and haemocidrosis (Fig. 14) other lesions appeared to be slight, the same as in lungs and lymph nodes where the splenic parenchyma showed granulomatous reaction (Fig. 15) which undergo caseation and calcification with infiltration by mononuclear cells and proliferation of fibrous connective tissue (Fig. 16). For farther diagnosis of the tubercle bacilli, spleen stained with Ziehl-Neelsen stain where the bacilli appeared as red road shape with blue black ground (Fig. 17).

Table (2): Correlation between post-mortem, bacteriological and pathological examinations of examined camels.

<table>
<thead>
<tr>
<th>Animal examined</th>
<th>Number</th>
<th>P/M</th>
<th>ELISA</th>
<th>Bacteriological examination</th>
<th>Pathological examination</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>%</td>
<td>No. (+ ve)</td>
<td>%</td>
<td>No. (b)</td>
</tr>
<tr>
<td>1st group</td>
<td>569</td>
<td>25</td>
<td>4.39(e)</td>
<td>8</td>
<td>32(d)</td>
</tr>
<tr>
<td>(Camel in contact with cattle)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2nd group</td>
<td>135</td>
<td>4</td>
<td>0.7(e)</td>
<td>1</td>
<td>25(d)</td>
</tr>
<tr>
<td>(Camel kept in closed farm)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>704</td>
<td>29</td>
<td>18.4(e)</td>
<td>9</td>
<td>1.3(e)</td>
</tr>
</tbody>
</table>

(a): Presence of tubercles like lesions.
(b) = 5 isolated microorganism identified as *Mycobacterium bovis*.
(c) = 5 presence of the typical tubercles granuloma with langerhan's gaint cells.
(d) = % calculated according to No. of P/M positive camels.
(e) = % calculated according No. of examined camels.
Fig. (1): Impression smear of lung showing red acid fast bacilli with dark blue back ground (Ziehl Neelson, X 650).

Fig. (2): Lung showing tuberculous pneumonia with large area of the alveoli was caseated and necrosis with thickening of the wall of pulmonary blood vessels and congested alveolar capillaries (H & E X 200).

Fig. (3): Lung showed hyperplastic proliferation of epithelial lining the bronchi (H & E X 400).

Fig. (4): Lung showed thickening of the pleura with fibrous connective tissues proliferation (H & E X 200).

Fig. (5): Lung showed typical tubercles lesions with caseous material in the central surrounded by aggregation of epithelioid cells and lymphocytes (H & E X 200).
Fig. (6): Lung showed calcified tubercle with mononuclear inflammatory cells with fibrous connective tissue reaction and scanty Langhans giant cells in the marginal zone. (H & E X 400).

Fig. (7): Lung showed old lesion, where most of the parenchyma and bronchii appeared fibrosed and bronchi surrounded by fibrous connective tissues with scanty lymphocytes (H & E X 400).

Fig. (8): Lung showed milliary tuberculosis where the lung appeared with different myriad tubercles all the same age and size sometimes calcification and infiltration by mononuclear cells were observed (H & E X 200).

Fig. (9): Lymph node showed severe haemorrhage (H & E X 200).
Fig. (10): Lymph node showed the beginning of the granuloma formation where clusters of neutrophils surrounding the invading bacilli (H & E X 400).

Fig. (11): lymph node showed whorl of epithelioid cells in the central of garnuloma that replaced the eneutrophils (H & E X 200).

Fig. (12): Lymph node showed granuloma with aggregation of epithelioid cells and lymphocytes (H & E X 400).

Fig. (13): Lymph node showed calcified tubercles surrounded with remnant of neutrophils, epithelioid cells and lymphocytes (H & E X 400).
Fig. (14): Spleen showed thickening in the wall of splenic blood vessels and haemocidrosis (H & E X 400).

Fig. (15): Spleen showed granuloma in the splenic parenchyme (H & E X 200).

Fig. (16): Spleen showed old granuloma with caseation, calcification and proliferation of fibrous connective tissues (H & E X 200).

Fig. (17): Spleen showed red rod shape tubercles bacilli (Ziehl-Neelsen X 400).
DISCUSSION

Tuberculosis is a chronic contagious disease caused by mycobacteria, which affects many vertebrate animals and particularly manifests itself in lungs and lymph nodes. The lesions are granulomas known as tubercles (Manefield and Timson, 1996).

From the result illustrated in table (2). It is clear that 5 animals from 569 camels kept in close contact with cattle were positive for tuberculosis while all examined camels kept in closed farm (group 2) were negative for tuberculosis with incidence of 0.7% and 100% bovine type, these were agreed with Refai (1993) who reported the incidence of tuberculosis in camels in Egypt by 0.3% to 4% and mostly of bovine type. The ELISA-PPD using protein labeled with horseradish peroxidase conjugate as a substitute for the specific anticanimal IgG conjugate, can be applied in the detection of antituberculosis antibodies in camels. ELISA could detect 9 cases as positive (1.3 %) but by comparison with bacteriological finding only 5 camels were positive from which *M. bovis* were isolated. The other false positive cases may be attributed to non specific antibodies due to other microorganism. These results coincided with El-Sergany et al. (1991) who failed to isolate Mycobacterium organisms from ELISA positive animals but the isolated organisms were *Corynebacterium, Staphylococci, Citrobacter species and E. coli*.

These positive animals by post mortem inspection and histopathological examination revealed typical tubercles granulomas mainly in lungs, lymph nodes and spleens with tubercules pneumonia and hyperplastic proliferation of cells lining bronchi which may be attributed to the irritation action of the microorganism thus the histopathological scores were generally positively correlated with CFU counts (Dormans et al., 2004).

In the early stages of active tuberculosis most tubercle bacilli are taken with macrophages that destroy them by various lysosomal enzymes and reactive oxygen intermediates ROI (Rook and Bloom, 1994). There are 3 strategies by which mycobacteria survive within macrophages. First, they inhibit fusion of the phagosome to the lysosomes. Second, they neutralize ROI by means of cell wall lipids including peptidoglycolipids and lipoarabinomannan and by secreting the enzyme super oxide disminase. Third, they escape from the phagosome and replicate in the cytoplasm of the cell (McDough et al., 1993). When tubercle bacilli enter macrophages and not destroyed, they replicated and kill the cell and local area of inflammation is established by attract
more phagocytes to the site of infection. Some bacilli transported within phagocytes to regional lymph nodes where they are engulfed by antigen presenting cells (APC) and the epitopes from mycobacteria presented on the cell surface formed (CD4 + helper T cells which undergo activation and produce cytokines including interferon Y (IFN-Y) that activates macrophages and draw more of these cells to the lesion arranged in compact palisade, resemble epithelial cells termed epithelioid cells that may fuse together to form multinucleated giant cells which are the characteristic of the granulomas of tuberculosis. This activated macrophages produce a cytokine termed tumor necrosis factor (TNFα) which plays a key role in protective immunity by maintaining the integrity of the granuloma and haematogenous spread occurs with implantation of bacilli in many organs leading to millary tuberculosis (Collier et al., 1998).

It is highly likely that the entire granuloma is much more capable of destroying tubercle bacilli than isolated macrophages. This palisade of metabolically active macrophages consumes oxygen diffusing into the granuloma so that the center became anoxic and undergoes necrosis termed caseation. This anoxia and free fatty acids provide an environment highly unfavorable to the bacilli many of which die and the granulome become inactive, fibroblasts surrounded them and may be calcified (Dannenberg, 1993). Our histopathological finding were in agreement with Wernery et al. (2007); Kinne et al. (2006) and Oevermann et al. (2004). All results agree with Gatt and Mack (1963) who reported that in Egypt, Tuberculosis didn't occur in nomadic camels but in those belonging to farmers who kept them in close contact with cattle. The mode of transmission of tuberculosis is unknown in camelids but it is presumed similar to that in cattle. In cattle it is mainly horizontal. It is believed that camelids suffering from pulmonary tuberculosis infect healthy animals via aerosols. The alimentary, congenital, veneral and cutaneous routes that may occur in cattle have not been described in camelid. Kogramanov et al. (1971) found that the Ixodes tick Hyalomma asiaticum can transmit M. tuberculosis to camels. In this concern Donchenko et al. (1975a) reported that tuberculosis is rare among camels kept under nomadic condition. The disease occurs more frequently when camels are kept in close quarters with other animals or in close contact with cattle.

It is concluded that the incidence of camels infected by T.B is 0.7% in Egypt. The infected camels were reared with cattle or beside cattle farms. The isolated
strain was *M. bovis*. The ELISA technique is most efficiency in the diagnosis of T.B as well as the histopathological examination.

REFERENCES


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بعض الدراسات على السل في الجمال
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المخصص العربي

أجريت هذه الدراسة على 400 جمل من بوق في المجازر المختلفة بمصر، وتم فحصهم بطريقاً مختلفاً لتشخيص مرض السل. قسمت هذه الحيوانات إلى مجسمتين: المجموعة الأولى مكونة من 549 جمل ربيت بالقرب من أبقار والمجموعة الثانية مكونة من 135 جمل الربيت في مزارع مختلفة.

وقد تم فحص كل من المجموعتين لوجود عقد سلية في جميع الأعضاء. وقد وجدت عقد سلية في 25 جمل من المجموعة الأولى معظمها في الرئة والثدي والليبافيا والطحال، بينما كان 8 جمال من هذه المجموعة إيجابية لإختبار الإلإزا. كما تم عزل ميكروب السل البقرى من عدد 5 جمال. وتم تشخيص السل بواسطة الفحص الهيستوباثولوجي الذي أسفر عن وجود عقد سلية خاصة بالسل بمراحل مختلفة في عدد 5 جمال من هذه المجموعة. أما المجموعة الثانية فقد وجد عدد 4 جمال إيجابي لوجود عقد سلية وعدد 1 جمال إيجابي لإختبار الإلإزا. بينما كانت كلها سالبة للفحص الميكروبيو بولوجي والهستوباثولوجي.

وقد تم تحديد نسبة الإصابة بالسل البقرى في الجمال بمصر بـ 7.2% خاصة في الجمال التي تربى بجوار الأبقار.

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