World Journal of Pharmaceutical and Life Sciences
WJPLS
www.wjpls.org
SJIF Impact Factor: 3.347

REVIEW ON CAMEL TUBERCULOSIS: DIAGNOSIS, PATHOLOGY AND PUBLIC HEALTH SIGNIFICANCE

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Article Received on 10/02/2016 Article Revised on 01/03/2016 Article Accepted on 22/03/2016

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ABSTRACT

Tuberculosis is a chronic, contagious, granulomatous disease caused by mycobacterial species belonging to the Mycobacterium tuberculosis complex. Camel tuberculosis has been diagnosed in many countries of the world since the beginning of 19th Century and it has worldwide distribution camelids were not considered highly susceptible to tuberculosis but in recent years increased numbers of cases have been experienced in some countries. In most of the cases transmission probably occurs through contact with infected cattle or wildlife. The occurrences of TB in camels relatively higher in the younger and older camels than other age groups. There are many potential sources of infection for came lids including airborne, droplets, feed contaminated with feces and urine of infected camel. Tuberculosis is spreads in the body of infected animals by two stages these are the primary complex and post primary disseminations. The organs most frequently affected in both groups of camelids are the lungs and associated thoracic lymph nodes where typical caseonecrotic lesions can be particularly extensive. Gross and microscopic lesions are important for the diagnosis of camel tuberculosis. None of the ante-mortem tests currently available can consistently provide accurate diagnosis of the infection in live camelids. Recently developed serological assays have the potential for rapid and accurate diagnosis of tuberculosis but still need to be validated. The clinical signs of TB in camelids include wasting, anorexia, respiratory distress, enlargement of superficial lymph nodes, decumbency, chronic weight loss or emaciation, weakness, dyspnea and cough eventually death. Control of TB is the subject of statutory regulation with culling of infected animals. Treatment of

infected animals is not usually attempttedal though; there are some reports of anti-TB drugs being used in captive wild animals. There are approximately 1415 pathogens known to affect humans of which about 61% of all human pathogens are zoonotic. Human tuberculosis of animal origin (zoonotic TB) is an important public health concern in developing countries. TB infection in human is principally caused by *M.tuberculosis* however; human TB animal origin caused by *M.bovis* is becoming increasingly prevalent.

KEYWORDS: *camel tuberculosis, pathology, diagnosis, zoonosis.*

1. INTRODUCTION

Camels were probably domesticated about 400 years ago. The camels were domesticated in southern Arabia Peninsula probably the area of Yemen and Oman. It has subsequently been distributed to the rest of the world (Wilson, 1998). It is the most important animal in the arid area of Africa particularly in the arid area low land of eastern Africai.e in Somalia, Sudan, Ethiopia, Kenya and Djibouti, approximately 11.5million camels in this region represents over 80% of the African and two third of the world's camel population (Dioli and Schwartz,1992). However, some dromedary keeping countries are located outside the tropics in North and South America, western Asia, India, Candy island, Caribbean, Italy and Southern Spain (Knoess,1977).

Tuberculosis occurs worldwide in people, wild and domesticated or captive animals (Krauss *et al.*, 2003).Tuberculosis is one of the major global reportable zoonotic diseases, killing approximately 1.5 to 2 million people every year (Thoen*et al.*, 2009). Although, *M.tuberculosis* is responsible for most human cases, bovine TB is caused by *M.bovis* an important zoonosis that can spread to people through ingestion of raw milk and sometimes by inhalation of infectious droplets. The disease had already been diagnosed around the turn of the century in dromedariesin Egypt (Wernery and Kaaden, 2002).Tuberculosis is a chronic, contagious, granulomatous disease caused by mycobacterial species belonging to the *Mycobacterium tuberculosis* complex (MTBC) (Thoen*et al.*,2006). Most of the members of this genus are independent saprophyte living in the soil, water and vegetation or in the mucus membrane of oropharynx but few are pathogenic to animal and man.

The disease in dromedaries has been documented since 1888. *Mycobacterium tuberculosis, Mycobacterium bovis, Mycobacterium kansasii, Mycobacterium aquae, Mycobacterium fortu itumand Mycobacterium smegmatis* have been isolated in camels as causative agents of camel

TB (Kinne*et al*., 2006). Camelids were not considered highly susceptible to TB (Fowler, 2010). But in recent years serious concern has arisen about TB in New World camelids (NWCs), particularlyllamas and alpacas, in some countries where they are reared (and not just countries in their native South America). For example, TB is a serious emerging disease inthe steadily increasing NWC population of the United Kingdom (UK), (Twomey*et al.*, 2010). Tuberculosis also affects Old World camelids (OWCs), including dromedaries and Bactrian camels (Mustafa I.E,1987). The disease affects many vertebrate animals and manifests particularly in lungs and lymph nodes but also in other organs.

The camel seems to be spared from the devastating epidemic infections which threaten other livestock species in the sameregion, e.g. Rinderpest, Contagious Pleuropneumonia and Foot and Mouth disease. The animal is however, affected by manyother diseases some of which are unknown to date. Cameldiseases that are shared with other species of livestock arecomparatively well-known while other camel-specific diseasesal though well-known to pastoralists for generations stillremain a mystery to the scientific community (Dirieand Abdurahman, 2003). Surveys conducted in Egypt, Sudan, India, Somalia and Kazakhstanhas shown that tuberculosis is rare, although some cases have been observed in Saudi Arabia, Chad and Burkina Faso. The commonest form is miliary or nodulartuberculosis of the lungs caused by *Mycobacterium bovis*(Fassi-Fehri,1987). Despite that, the camel may contract many other diseases some of which are still unknown (Chandel and Kher, 1999).Recently, few studies conducted in the epidemiological investigation of Tuberculosis in camels (Gezahegn *et al.*,2011).Thus, the aim of this review is to highlight the importance of Camel Tuberculosis with particular emphasis on Pathology, diagnostic alternatives and public health implication.

2. LITERATURE REVIEW

2.1. Etiology

The causative agent of camel tuberculosis is the genus *Mycobacterium* of the family *Mycobacteriaceae* includes gram positive non-motile pleomorphic bacilli and non-sporing acid-fast rods of various lengths (Quinn*et al.*, 1994). Mycobacteria possess a waxy coat that makes it difficult for the host's defense mechanisms to destroy them and results in a slow chronic disease (Taylor *et al*., 2007).

Although, the extent of tuberculosis has been well documented in humans and most domestic animals, very little is known about the pathology and cause of camel TB in pastoral areas of

the world. The following species are grouped in the Mycobacterium Tuberculosis Complex (MTBC): *M. tuberculosis, M. canettii, M. africanum, M. bovis, M. pinnipedii, M. caprae* and *M. microti* (Thoen*et al.*, 2009). The causative agents of TB in dromedaries are broadly classified as typical and atypical. The two most common typical mycobacteria are *M.bovis* and *M.tuberculosis*, the atypical species of mycobacteria cause disease in the camel when it becomes immunocompromized (Allen *et al*.,1992). Some of the atypical *Mycobacteria* rarely causing TB in camels are *M.kansasii, M.aqvae, M. smegmatis, M.microti* and *M.fortuitum.*

The main cause of camel TB is *M. bovis* and *M. tuberculosis,* a small, aerobic, nonmotilebacillus (Dolin *et al*., 2010). The high lipid content of *M. tuberculosis* accounts for many of its unique clinical characteristics (Southwick, 2007). It divides every 16 to 20 hours, which is an extremely slow rate compared with other bacteria, which usually divide in less than an hour. Mycobacterium has an outer membrane lipid bilayer. If a Gram stain is performed, MTB Ceither stains very weakly gram-positive or does not retain dye as a result of the high lipid and mycolic acid content of its cell wall (Madison, 2001).MTBC can withstand weak disinfectants and survive in a dry state for weeks. In nature, the bacterium can grow only within the cells of a host organism, but *M. tuberculosis, M.bovis, M.microti, M.fortuitum, M.kansasii and M.smegmatis* can be culturedin the laboratory (Parish*et al.*, 1999).

2.2. Epidemiology

There is little published information on the epidemiology of tuberculosis specifically relating to camelids. In the Egyptian Official Gazette in 1888, only sporadic reports were documented until Masonin 1912, published his pathological observations on a series of 20 cases detected during a year's surveillance at Cairo abattoir (Mason,1912). In 1987 Mustafa mentioned in a brief review that disease was more commonly observed in farmed camels and those in close proximity to cattle but appeared to be rare among nomadic camels, suggesting that close contact facilitates transmission between domesticated animals.

2.2.1. Geographical distribution

Camel tuberculosis has been diagnosed in many countries of the world since the beginning of 19th Century and it has worldwide distribution and relatively rare in Somalia (Abdurahman and Bornstein, 1991), the country which at that time had one of the largest populations of

OWCs in the world (Teka, 1991). Surveys conducted in Egypt, Sudan, India, Somalia and Kazakhstan has shown that tuberculosis is rare although, some cases have been observedin Saudi Arabia, Chad and Burkina Faso. The commonest form is miliary or nodulartuberculosis of the lungs caused by *Mycobacterium bovis*(Richard, 1979).

A recent study in Ethiopian abattoirs has suggested a prevalence of 10% based on the identification of gross lesions in 906 apparently healthy camels (Mamo*et al*., 2011). Among animals with suspicious lesions,Mycobacteria were cultured from 31 of 91 animals but only two of theseisolates were MTBC bacteria, both *M. bovis*and *M.tuberculosis*.

The prevalence of tuberculosis in dromedaries is also rare in Dubai, where only four cases have been seen in a 25-year observation period (Wernery *et al*., 2007). Although NWCs were once considered not very susceptible to TB (Fowler, 2010), many cases have been reported in recent years, some associated with high morbidity (Dinkla*et al.*,1991). Onereason for this increase is that NWCs are increasingly beingkept in areas where TB is endemic (Connolly *et al*., 2008).

2.2.2. Susceptible hosts

Mycobacterium tuberculosis complex has a broad host range. The host range includes free ranging wildlife, captive wildlife, domestic livestock, non-human primate and humans (Biet*et al*., 2005). Susceptible domestic species includes camel, cattle, goat, sheep, dogs, pig, equine, cat and Ilama (O'Relly and Daborn, 1995; Jahans and Worth,2006).

2.2.3. Age and sex

The occurrence of TB in camels is relatively higher in the younger and older camels than other age groups. Other researchers have also reported in cattle particularly that older animals are affected by TB (Kazwala et *al*., 2001) which could be due to the fact that older animals have weaker immune system. The higher frequency of TB in younger camels could be due to the less developed immunity (Menzies and Neill, 2000). It was observed that TB lesion was more frequently observed in female camels as compared to male camels. This could be due to the fact that female camels are brought for slaughter at their older age after completion of the reproductive age (Munyeme*et al*., 2008).

2.2.4. Source of infection

Mycobacteria are generally not species-specific pathogens (Twomey*et al*., 2010). Inter-species transmission may therefore occur and there are many potential sources of infection for camelids. *M. bovis*strains isolated from NWCs are often the same molecular types that are isolated from tuberculous cattle and badgers in the same geographical area suggesting spillover of infection from non-camelid reservoirs (Twomey*et al*., 2009). This presents a challenge for control if contact with the reservoir cannot be avoided. Badgers, for example, are an important wildlife reservoir for bovine TB and are known to visit farm buildings and food stores, which they contaminate with feces and urine potentially transmitting *M. bovis*to other animals (Roper *et al.,* 2003). In a rare case of *M. pinnipedii*infection affecting an OWC, the most probable source was a sea lion kept in the same zoo (Moser *et al.,* 2008).

2.2.5. Mode of spread and transmission

Once infected, a camelid can introduce the disease into a non-infected herd with subsequent spread to other camelids. There are different modes of spread of tuberculosis between camel herds (Bush *et al.*, 1990). It is believed that camels suffering from pulmonary tuberculosis infected healthy animals via aerosols, alimentary, congenital,venereal and cutaneous route that may occur in cattle have not been described in camels. Ixodes tick *Hayalomaasiaticum*can transmit *M. tuberculosis* to bacterianus camels (Kogramanove*et al.*,1991). Young camels can also be easily infected with higher doses of mycobacteria via colostrum's from infected camel in a similar way, as it occurs in cattle (Phillips *et al.,* 2003). In connection with this, another report mentioned of vertical transmission of *M. bovis* from an infected dam to her calf through congenital infection in utero (Ozyigit*et al*., 2007).

2.3. Pathogenesis

Tuberculosis is spreads in the body of infected animals by two stages these are the primary complex and post primary disseminations. The primary complex characterized by the lesion at the point of entry and in the local lymph node. The lesion at the point of entry (mainly in the respiratory tissue) is common when infection is by inhalations. When infection occurs via the alimentary tract, the lesion at the site of entry is unusual although tonsillar and intestinal ulcer may occur. In the alimentary infection, the more common observable lesion is the pharyngeal or mesenteric lymph node (Carter,1984).

The extent and sizes of tuberculosis lesions can vary. Lesions are found in the lung,liver, various organs, lymph node,and the skin but are rarely seen in muscles(Dungworth,1985). A

visible primary focus develops within eight days of entry being infected by bacteria. Calcification of the lesion commence about two weeks later. The developing necrotic focus is soon surrounded by granulomatous tissue and lymphocyte and the pathgnomictubercule or granuloma is established. The bacteria pass from this primary focus which is in the respiratory tract in 90-95% of cases to regional lymph node and cause the development of similar lesion there.Post primary dissemination from the primary complex may take the form of acute miliary tuberculosis, discrete nodular lesion in various organs, or chronic organ tuberculosis caused by endogenous or exogenous re-infection of tissue rendered allergic to PPD (Radostits*et al*., 1994).

2.4. Clinical Signs

Tuberculosis is a chronic debilitating disease. The clinical signs in camelids include wasting, anorexia, respiratory distress, enlargement of superficial lymph nodes, decumbency, chronic weight loss or emaciation, weakness, dyspnea cough eventually death (Abdurahman, and Bornstein, 1991) Clinical signs are often associated with extensive respiratory pathology and it is surprising that overt respiratory distress is sometimes not observed in animals with severe lung lesions (Dinkla*et al.*,1991). Animals are occasionally found dead with no previous clinical observations (Barlow *et al.*, 1999). Unfortunately many infected camels are asymptomatic until disease is advanced. Therefore, protective quarantine and routine screening program should be developed for each zoologic collection housing susceptible species (Twomey, 2011).

2.5. Pathology

The pathology of tuberculosis in old world camels (OWCs)was described nearly 100 years ago in cases detected in an Egyptian abattoir (Mason, 1917)**.** Pathological reports on new world camels (NWCs) have also emerged in recent years (Ryan E.G,*et al.*, 2008). The organs most frequently affected in both groups of camelids are the lungs and associated thoracic lymph nodes where typical caseosnecrotic lesions can be particularly extensive. Mason (1912) mentioned that all cases in OWCs had lesions at these sites, 60% of cases exclusively involving these sites other affected tissues included the liver, spleen, kidney, tracheaand pericardium. A similar distribution of lesions has been reported in NWCs (Twomey*et al.*, 2007). Intestinal and cutaneous lesions have also been observed in llamas (Twomey*et al.*, 2010).

2.5.1. Gross Lesions

Gross lesions consisted of multiple confluent, yellowish, caseous nodules (diameters up to 10 cm) with friable centers in the lungs, livers, spleen, bronchial lymph nodes, hepatic lymph nodes, mediastinal lymph nodes, and mesenteric lymph nodes. Similar nodules of a smaller size are observed in the adjacent serosa. By examination of cut sections, these nodules are yellowish and firm with an onionskin-like structure and a partially mineralized center. Additional findings included hydrothorax, ascites, hepatic lipidosis, splenomegaly, pulmonary edema and congestion, esophageal petechiae, intestinal hemorrhages and cervical subcutaneous edema (Kamerbeek*et al*., 1997).

The severity of pathological descriptions varies depending on how animals are selected for necropsy. Thus, animals selected following clinical disease often have severe pathology whereas infected animals identified in ante-mortem immunological tests and culled prior to development of clinical signs are likely to have less severe gross lesions (Werner*yet al.*, 2007).

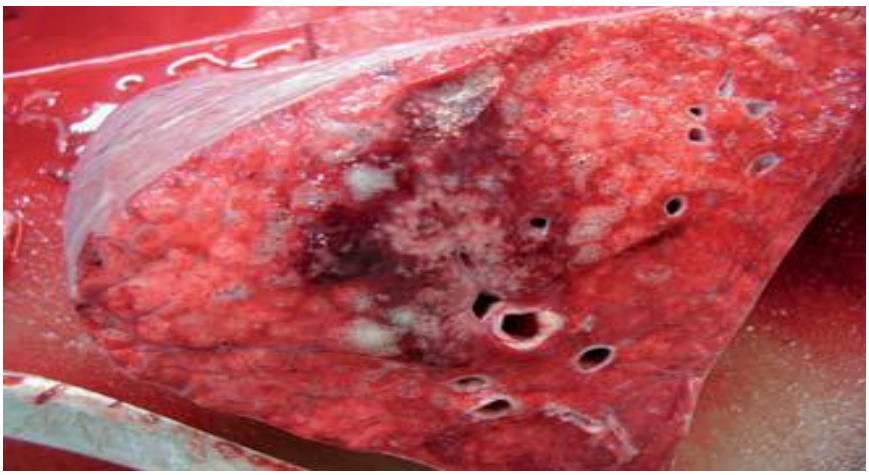

Figure1: Cut surface of a lung of a seropositive camel with central grey area due to tuberculous granulomas (Wernery *et al*., 2007).

2.5.2. Microscopic Lesions

Histologically, the caseous nodules presented as granulomas composed of large numbers of closely packed epithelioid macrophages are mixed with various numbers of lymphocytes, plasma cells, and neutrophils. The larger granulomas showed central necrosis with foci of mineralization and fibrous capsules of various thicknesses. Epithelioidmacrophages contained eosinophilic and fine granular cytoplasms. Ziehl-Neelsen and Fite-Faraco staining revealed abundant acid-fast bacilli (AFB) within the epithelioid macrophages throughout all

layers of the granulomas. They appeared as irregular long and straight or curved structures without branches **(**Kremer *et al*., 1998).

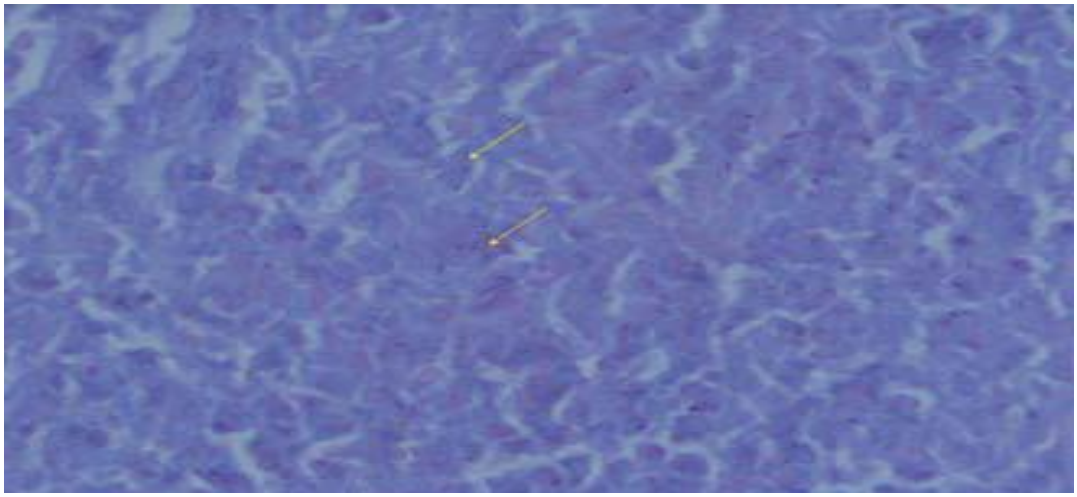

Figure 2: Histopathology of the same camel lung with granulomas and single acid-fast rods (arrows) in epithelioid cells (Ziehl-Neelsen staining). (García-Bocanegra*et al*., 2010).

2.6. Diagnosis

Diagnosis of tuberculosishas been developed since the 1980s and potentially offers a convenient and cost-effective means of TB surveillance. Early attempts at developing enzyme linked immune sorbent assays (ELISAs) did not convincingly discriminate between naturally infected and vaccinated animals and issues of cross-reactivity with other mycobacteria were not addressed (Dean *et al.*, 2009). Diagnosis of tuberculosis infection in camels is often based on clinical signs, necropsy findings and specific immune response detection and removal or slaughter of infected animals and/or herds. In camelids, this strategy is difficult to conduct because of the lack of adequate tests for live animals (Alvarez *et al.*, 2011).

A definitive diagnosis can be made only at post-mortem examination by demonstration of typical gross lesions followed by histopathology and confirmatory bacterial culture. Mycobacteria are slow-growing organisms that may require incubation on selective media such as Lowenstein-Jensen or Ogawa for up to eight weeks (Tanoue *et al.*, 2002). Because of the chronic nature of the disease and the multiplicity of signs caused by the variable localization of the infection the disease occurs in a particular area it must be considered in the differential diagnosis of many other diseases (Radostits*et al*., 1994).

The diagnosis oftuberculosis in the living animals mainly based on the tuberculin skin test and demonstration of the organism in exudates or excretions from lesions of slaughtered animals. Diagnosis of TB in live camelids faces many difficulties (Wernery*etal.*, 2002).

2.6.1. Physical Examination

Physical examinations of the studied camels carried out before slaughtered. Body temperature, pulse rate, respiratory rate, and type of nasal discharge if present, condition of regional lymph nodes and visible mucous membranes were examined and recorded for individual animal (Fowler, 1998).

2.6.2. Post Mortem Examination

Postmortem inspection is performed following the procedure as described by (Corner, 1994). Mandibular, retropharyngeal, bronchial, mediastinal, mesenteric and hepatic lymph nodes are examined and organs including lungs, liver, small intestine and kidneys should be examined in detail during post-mortem in the abattoir under a bright-light source. The lobes of the left and right lungs are inspected and palpated externally. Then, each lobe is sectioned into about 2-cm-thick slices to facilitate the detection of lesions with sterile surgical blades. Similarly, lymph nodes are sliced into thin sections (about 2mm thick) and inspected for the presence of visible lesions. Whenever gross lesions suggestive of TB are detected in any of the tissue the tissue is classified as having lesions(Corner, 1994).

2.6.3. Tuberculin Test

Two types of the skin test are used for the diagnosis of camel tuberculosis, the single intra dermal tuberculin test (SITT) and the single comparative intradermal tuberculin test (CITT). Tuberculin is a product containing purified Protein Derivatives (PPD) prepared from M. *bovis*, *M.tuberculosis* or *M.avium* that is bovine, human or avian tuberculin respectively. For the diagnosis of TB in camels bovine PPD and avian PPD will be injected (0.1ml) in to a shaved and marked area. The two PPD will be at two different skin locations about 12 cm apart. The sites utilized for evaluating the use of tuberculin test in camel are middle of the neck, auxiliary site and under the tail (only for bovine PPD in case of SITT).

According to Wernery and his colleague study for SITT the skin thickness is measured before and after tuberculin administrationand the difference are recorded at days 2, 3, 5and 7. The difference in skin thickness of<2.0 mm is considered negative from 2.0 to 4.0 mm is suspected and >4.0 mm is reactors (Wernery *et al*., 2007). On the other hand for CITT, the

difference between the reactivity to bovine PPD and avian PPD are recorded for each injection site at days 2, 3, 5 and 7. The CITT is considered positive if the reaction to bovine PPD is>4.0mm greater than that of avian PPD in conclusive if it is only from 1.o to4.0mm greater and negative result is recorded if the reaction to bovine PPD is equal to or smaller than that obtained at the avian site of injection (Wernery *et al.*,2007). The most potent reaction in camel are found in both skin test type when using axillary injection and reading reaction five days later (Werrnery and Kaaden, 2002).

2.6.4. Animal Inoculation

Animal inoculation is now rarely performed because of aesthetic and economic reason as well as the risk of infection to laboratory staff which requires biologically safe animal facility (Quinn,1994).To probe for tuberculous mycobacteria that cause disease in camels a guinea pig will be injected subcutaneously in to the left flank with 0.2ml of crushed lung tissue of infected dromedary. After inoculation 20 days later the guinea pig will be examined for the development of any primary tuberculous lesion in different parts of its body including liver, spleen, kidney and lung. In addition histopathological examination and acid fast staining of the lesions can be used to observe the granulomatous lesion with acid fast bacilli (Kinne *et al*.,2006).

Table 1: Inoculations of laboratory animal with mycobacteria of the tuberculosis group and type of lesions developed.

	M. tuberculosis	***M. bovis***	***M. avium***
Rabbit(intravenously) (generalized)	+-(pulmonary only)	++(miliary)	++++
Guinea-pig	++(generalized)	++(generalized)	(-+)(focal)
Chicken (intravenously)	--	--	++

Source (Kinne, *et al*., 2006)

2.6.5. Isolations of mycobacteria

Acid-fast stain (Ziehl-Neelsen's) stain

The mycobacteria cell wall contains a waxy coat known as mycolicacid which retains the primary staining dye, basic fuchsin (Cater, 1984). The acid-fast stain is used to differentiate microorganisms which resist decolonization with alcohol solution as their cell wall contains a waxy coat the procedure of acid- fast stain take suspicious organ sample from camel that died due to tuberculosis and stain with Ziehl-Neelsen (ZN). The sample are treated with concentrated carbon fuchsin and heat at interval until steam rise without boiling the fluid,

wash in water, decolorize by flooding the slide with acid-alcohol for 1-2 minutes and wash in water, counter stain with methylene blue or malachite green for 30-60 seconds wash in water, blot dry and examined under oil immersion, the acid-fast mycobacteria retain the carbon fuchsin and appear as bright red or pink with blue or green back ground (Carter, 1984).

Culturing of mycobacterium

Culture of *M. bovis* from lung and lymph node using Lowenstein-Jensen (JL) medium slants after decontamination and concentration of the sample (Tell *et al.,* 2003) is the gold standard method of diagnosis of tuberculosis.The samples are further processed for isolation of mycobacteria in accordance with the Office International des Epizooties(Ameni*et al*., 2007). The specimens are sectioned using sterile blades minced with scissors and homogenized with a sterile mortar and pestle under a biological safety cabinet. The homogenates are decontaminated by adding an equal volume of 4% Noah on the sample in order to remove contaminants. Thereafter, centrifuged at 3,000 rpm for 15 minutes to concentrate the mycobacteria. The supernatant is discarded and the sediment is neutralized by 1% (0.1 N) HCl acid using phenol red as an indicator.

Neutralization is achieved when the color of the solution changed from purple to yellow (OIE, 2004). Next, 0.1 ml of suspension from each sample is spread onto a slant of Lowenstein Jensen (LJ) medium. An agar-based medium such as middle brook 7H10 and 7H11 or blood based agar medium may also be used (OIE, 2009). The media are prepared as solid slants in screw-capped bottles. Duplicate slants are used, one enriched with sodium pyruvate and the other enriched with glycerol. Glycerol is inhibitory to *Mycobacterium bovis*while sodium pyruvate (0.4%) enhances its growth. Each new batch of culture medium should be inoculated with the stock strains of *Mycobacteria* to ensure that the medium supports satisfactory growth (Quinn, 1994). Thus, the media with glycerol and without glycerol (but with sodium pyruvate) should be inoculated. Malachite green dye (0.025g/100ml) is commonly used as selective agent.

The media can be made more selective by the addition of cycloheximide (400µg/ml), lincomycin (2µg/ml) and nalidixic acid (35µg/ml). The inoculated media may have to be incubated at 37°C for up to 8 weeks and preferably for 10 to 12 weeks with or without carbon dioxide for the mycobacteria in the tuberculosis group (OIE, 2009). *Mycobacterium tuberculosis* and *Mycobacterium avium* prefer the caps on the culture media to be loose, while *Mycobacterium bovis*grows best in airtight containers (Quinn and Markey, 2003).

Colonial Morphology:The luxuriant growth of *Mycobacterium tuberculosis* on glycerol containing media, giving the characteristic 'rough, tough and buff colonies is known as eugenic while the growth of *Mycobacterium avium* on media containing glycerol is also described as eugenic. *Mycobacterium bovis*has sparse, thin growth on glycerol containing media that is called dysgenic. *Mycobacterium bovis*, however, grow well on pyruvate-containing media without glycerol (Patterson and Grooms, 2000).

2.6.6. Serological Tests

With none of the currently available tests being able to detect the disease with certainty antibody blood assay for TB have not been extensively evaluated in camelsalthough, in other host species serological approaches proved to be useful (Greenwold*et al.*,2003).

Enzyme linked immunoabsorbent assay (ELISA) technique

Enzyme Linked Immunoabsorbent Assay (ELISA) for serological diagnosis of tuberculosis in camels is performed as described by (Rilacco*et al.,* 1990). Microtitre plates are coated with bovine 50 µlPPD diluted in (10 µg/ml) carbonate bicarbonate buffer (pH 9.6) and incubated for 20 hrs at 4°C in humfied atmosphere. After washing with PBS tween 20 (0.05% w/ v) wells are blocked 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 are added and incubated at 37 °C for 60 minutes. After 3 washing with PBS (pH 7.4) containing 0.01% tween 20, 150 µlof protein A horseradish peroxidase conjugate (1:1000) is added to each well and then incubated at 37 °C for 60 minutes. After washing again working solution of ABTSsubstrate (100 µl/well) is added and incubate at 37 °C for 15 minutes.

The optical density recorded at 405 nm in Dynatech micro- ELISA reader. An ELISA reading that is equal to/or higher than double fold of the ELISA reading of negative control is considered positive.More recent serological tests include the multi-antigen print immunoassay (MAPIA) and the Vet TB Stat-Pak or 'rapid test' (Waters*et al*.,2006).

Multi-antigen print immunoassay (MAPIA)

The Multi-antigen print immunoassay(MAPIA)utilises a range of antigens printed onto nitrocellulose strips that are incubated withSerum samples. A panel of mycobacterium antigens including 8 purified recombinant proteins (ESAT-6, CFP 10, Acr 1, 38 KDa protein, MPB59, MPB64, MPB 70 and MPB83), 2 protein fusion (CFP-10/ESAT-6 and Acr1/ MPB

83), 2 native antigens, bovine purified protein derivatives (PPD) and *M. bovis* culture filtrate can be used.Antigens are immobilized on nitrocellulose membrane at a protein concentration of 0.05 mg/ml by using a semiautomatic microaerosolization device to generate invisible parallel bands. After antigen printing the membrane will be cut into strips three mm wide perpendicular to the antigen bands, so that each strip carried all antigen. Strips will be blocked for one hour with 1% non-fat skimmed bovine milk in phosphate-buffered saline containing 0.05 % Tween -29 and then incubated with individual serum samples diluted 1:50 in blocking solution for 1 hour at room temperature. After washing strips will be incubated for one hour with peroxidase conjugated protein G (Sigma) diluted 1:100 followed by another washing step. Immunoglobulin G antibodies bound to immobilized antigens will be visualized with 3, 3, 5, 5 tetra methyl benziudine. MAPIA results will be scored and a band of any intensity being read as a positive reaction (Lyashchenko*et al*., 2006).

Vet TB Stat-Pak or rapid test

Rapid test is a portable lateral-flow chromatographic assay that uses three MTC-specific antigens. These antigens have shown some promise for detecting MTC-infected camelids, but further validation is still required before they can be used reliably for field diagnosis (Lyashchenko *et al*., 2007).

The test employs a cock tail of selected *M. bovis*and *M. tuberculosis* antigens (that have been isolated from the cell wall, cytoplasm or culture filtrate) and blue latex base bead –based signal detection system. Various mycobacterium antigens have been isolated from the cell wall, cytoplasm or culture filtrate and are immunologically active as antigens, haptens and adjuvants. A disposable device consists of a plastic cassette containing a strip of cellulose membrane impregnated with test antigen and laminated with several pads made of glass fiber and celluose is used. The test requires 30 micro litter of serum sample and three drops of sample diluents that are added sequentially to the sample pads. As diluted test sample migrates to the conjugate pad, antigen conjugated latex particle bind anti body, if present in the sample, thus creating colored immune complex.

Derived by capillary forces this complex flow laterally across the nitrocellulose membrane and binds to the immobilized antigen thus producing a visible blue band in the test area of the device (Barksdale and Kim, 1994).In the absence of specific antibody no band develops in the test window. The liquid continues to migrate along the membrane producing a similar blue band in the control area of the device irrespective of the presence of specific antibody in

the test sample demonstrating that the test reagents are functional properly. Results are red after 20 minutes. Any visible band in the test areain addition in the control lineis considered as an antibody positive result whereas no band in the test area in addition to in the visible control line is considered as an antigen negative result (Lyashchenko*et al.*, 2006).

2.6.7. Histopathological examination

Post-mortem examination is carried out on the slaughtered camels. Tissue specimens are 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 are stained with haematoxylin and eosin and examined microscopically for the detection of acid fast bacilli in tissues sections are stained with Ziehl-Neelsen stain according to (Bancroft *et al.*, 1996).

2.6.8. Molecular diagnostic tests

Polymerase chain reaction (PCR)

Polymerase Chain Reaction (PCR) is a powerful tool that is used in a wide variety of diagnostic procedures. The PCR is used to detect the presence of genetic material deoxyribonucleic acid (DNA) that is unique and specific to an organism of interest. PCR works by amplifying a portion of DNA that is specific for that organism. This product can be easily visualized using standard laboratory procedures. The PCR test is very sensitive and can detect the presence of an organism when present at very low levels (OIE, 2009). PCR methods allow direct identification of the *Mycobacterium tuberculosis complex* and can detect less than 10 bacteria in a clinical specimen. PCR sensitivity ranges from 70 - 90% compared to the results of culture and its specificity varies between 90 and 95%. In smear of positive cases the sensitivity of PCR is greater than 95%but in smear of negative cases, it is only 50 to 60%. Therefore, at present amplification methods should not replace diagnostic conventional culture (Sharma and Gupta, 2011).

For the diagnosis of camel tuberculosis PCR is used to identify *M. bovis*in tissue collected at necropsy from animals suspected of being infected with camel tuberculosis. PCR is the only used on tissue that have histological (microscopic) evidence compatible with camel tuberculosis. The result can typically be obtained within 7 days and are classified as either positive or negative. A positive test obtained on PCR is highly suggestive that the animal is infected with camel tuberculosis. PCR has been widely evaluated for the detection of Mycobacterium tuberculosis complex (MTC) in clinical samples mainly sputum in human

patients and has recently been used for the diagnosis of tuberculosis in animals (Gori*et al*., 2005).

Spoligotyping

Spoligotyping also called space oligonucleotides typing is a novel or new method for simultaneously detection and typing of mycobacterium tuberculosis complex bacteria has been recently developed. This method is based on polymerase chain reaction (PCR) amplification of highly polymorphic direct repeat (DR) locus in the *M. tuberculosis* genome. The DR region in *M. bovis*contains direct repeat sequences of 36 whichare interspersed by the non-repetitive DNA spacers of 35 – 41 in length. Other MTC strains contain one or more IS6110 elements in DR-region (Sharma and Gupta, 2011).

Spoligotyping applied to culture is simple robust and highly reproducible (Soolingen, 2008). Results can be obtained from*M. tuberculosis* culture within one day. Thus, the clinical usefulness of spoligotypingis determined by its rapidity both in detecting causative bacteria and in providing epidemiologic information on strain identities. It can also be useful for identification of outbreak and can facilitate contact tracing of tuberculosis. PCR based methods are available as diagnostic and confirmatory test for tuberculosis and are expected to detect as low as 1 to 10 organisms (Gori*et al*., 2005).

Implementation of such a method in a clinic setting would be useful in surveillance of tuberculosis transmission and intervention to prevention of this disease (Gori*et al*., 2005). The specificity and sensitivity of this technique has been found to be 98 and 96% respectively with the clinical samples (Sharma and Gupta, 2011). One of the clearest advantages of Spoligotypingover IS6110 RFLP typing is that in principlespoligotyping can be used simultaneously for the detection and typing of MTC bacteria in one assay and requires viable organisms (Soolingen, 2008).

Restriction Fragment Length Polymorphism (RFLP)

Thisis considered as a gold standard for the molecular typing of *M. tuberculosis* due to its high discriminative power and reproducibility. It can also be used for outbreaks identification and can facilitate contact tracing of tuberculosis (Sharma and Gupta, 2011). However, this technique requires large amount of DNA and is therefore restricted to the mycobacterial cultures which take around 20 to 40 days to obtain sufficient DNA needed and for the combine process of probe labeling DNA fragmentation, electrophoresis, blotting,

hybridization, washing and auto radiograph. Moreover, this technique is also technically demanding, slow, cumbersomeand expensive and requires sophisticated analysis software for result analysis (Patterson and Grooms, 2000).

2.7. Public Health Significance of Camel TB

World Health Organization (WHO) defines zoonosis as those diseases and infections which are naturally transmitted between vertebrate animals and man. There are approximately 1415 pathogens known to affect humans of which about 61% of all human pathogens are zoonotic (Anon, 2011d). Nearly half of all human infectious diseases known today can be classified as emerging and about 75% of emerging infectious diseases are caused by zoonotic pathogens(Anon,2011).

Human population encounters animal disease with varying frequency depending on their occupation geographical location and the prevailing culture of the country. Weather living in urban or rural environment animals constantly may have close contact with human on farm (food producing animals) at area of residence (dogs, cats, cage birds) through leisure activities (horse, wild life) or by virtue of the occupation of individual as veterinarians or animal nurses. This close contact can result in the occurrence and transmission of zoonotic disease which is naturally transmitted between vertebrate animal and man. Zoonotic tuberculosis is an infectious disease of domestic animals that can be transmitted from animal to human through consumption of raw milk and meat from infected animals and directly through erogenous route (Quinn *et al*., 1994).

Tuberculosis is one of the major global reportable zoonotic diseases killing approximately 1.5 to 2 million people every year (Thoen et *al*.,2009). Although,*M. tuberculosis* is responsible for most human cases bovine TB caused by *M. bovis* is an important zoonosis that can spread to people throughingestion of raw milk and sometimes by inhalation of infectious droplets (Thoen*et al*.,2006). Outbreaks of bovine TB are therefore of considerable concern to public health officials and personnel responsible for the health of animals in zoos animal parks and private herds (Moser*et al*.,2008). The number of *M. bovis*-associated human TB cases has significantly declined in developed countries as a result of eradication programmes and pasteurization of milk (Thoen*et al*.,2006). Nevertheless, *M. bovis*still represents a zoonotic risk for people who are in close contact with infected animals for example a case of cutaneous TB in a veterinary surgeon was associated with contact with an infected alpaca (Twomey*et al*.,2010). Camel milk is also a potential source of infection particularly as it is

commonly consumed without boiling and *M. bovis*was isolated from pooled milk samples from camels in Russia (Donchenko*et al*.,1975).

Human tuberculosis of animal origin (zoonotic TB) is an important public health concern in developing countries. TB infection in human is principally caused by *M. tuberculosis*howeverhuman TB animal origin caused by *M.bovis* is becoming increasingly prevalent (Bedard*et al.,* 1993). Due to the lack of both control and diagnostic measures and pasteurization of milk coupled with a high prevalence and incidence of HIV/AIDS in human population(Cosivi*et al.*,1998).Concomitantly,TB is a major opportunistic infection in HIV-infected persons,and the world health organizations (WHO) estimated that 70%(6 million) of the people co-infected with TB and HIV. The prevalence, incidence and death caused by TB reported in 2002 by WHO (**Table 2**).

Table 2: Worldwide TB Figures.

World population		6219000000
TB infected people		2073000000
Estimated incidence	Tuberculosis Pulmonary TB	8797000 3887000
Death due to TB		1800000
TB case due to HIV		9%
Death of TB patient due to HIV		12%

Source (WHO, 2002)

2.8. Treatment, Control and prevention

Tuberculosis is a reportable disease in many countries and where this is the case control is the subject of statutory regulation with culling of infected animals. Treatment of infected animals is therefore not usually attempted although, there are some reports of anti-TB drugs being used in captive wild animals (Thoen*et al.*,2009). After the diagnosis of TB in Bactrian camels kept in a zoo prophylactic treatment of the remaining camels is attempted using isoniazid incorporated into pelleted feed at a dose of 2.4 mg/kg, fed *ad libitum* (Bush*et al*., 1990). However, possibly due to isoniazid toxicity several camels died exhibiting signs of bone marrow suppression. National control programmes are often based on intradermal tuberculin testing but because of the limitations of this test in camelids these programmes are unlikely to be successful however, a combination of ante mortem assays could improve the sensitivity of herd testing. Control depends on the removal of infected animals and prevention of further introduction of infection into the herd, but the disease will not be eradicated until infection is

controlled in reservoir hosts, such as in wildlife (Thoen*et al*.,2006). Vaccines are not yet available for camelids.

3. CONCLUSION AND RECOMMENDATIONS

Tuberculosis is a disease that has already been diagnosed around the turn of century in dromedaries. It is chronic, contagious, granulomatous disease caused by mycobacterial species belonging to the *Mycobacterium tuberculosis* complex. Most of the members of this genus are independent saprophytes living in the soil, water and vegetation or in the mucus membrane of oropharynx but few are pathogenic to animals and man. The organs most frequently affected are the lungs and associated thoracic lymph nodes, where typical caseousnecrotic lesions can be particularly extensive.Diagnosis oftuberculosis infection in camels is often based on clinical signs, necropsy findings,tuberculin testing and specific immune response detection.Studies done indifferent region of the world indicated clearly that the disease has a significance effect both in animals and humans in area where control of the disease is not implemented and consumption of uncooked product of camels is adopted.Nomadic people who are closely tied with rearing of camels are at risk of being infected with zoonotic camel TB and they contract the disease mainly through consumption of raw milk and other animal products. In addition, people having close association with infected animals have high probability of acquiring the infection. In general camel tuberculosis requires a good deal of emphasis from the point of its economic and zoonotic significance especially in countrieswhere camel has special cultural and economic importance.

Based on the above conclusion, the following recommendations are forwarded:

- The government should enforce the implementation of routine meat inspection in all camel slaughter houses found in the country particularly focusing for detection of infected camels in line with cattle.
- Close confinement of camel with cattle should be minimized wherever possibleand segregation of camels based on age especially young and old age should be practiced.
- The veterinarians should understand the pathology of camel tuberculosis and perform correct diagnosis for effective control and prevention of camel tuberculosis.
- Further epidemiological investigation of camel TB need to be conducted specially to the molecular level.

- The government and concerned bodies should give more emphasis on creating awareness about camel tuberculosis and its zoonotic importance among the camel rearing community and meat inspectors.
- The people especially in the pastoral area must drink pasteurized camel milk and cooked meat.

4. ACKNOWLEDGEMENT

I would like to express my great, heart-felt, gratitude and respect to my academic advisors their intellectual advice and unreserved guidance.

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