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The possible role of cattle and buffaloes in transmitting *Mycobacterium tuberculosis* to man.

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Abstract

Mycobacterium tuberculosis is a human pathogen, but recent studies recorded the presence of *M. tuberculosis* in domestic animals. This work was carried out to investigate the possible role of cattle and buffaloes in the epidemiology of *M. tuberculosis.* For this purpose, 68 tuberculous tissue samples were collected from cattle and buffaloes from different organs at different slaughterhouses in Minoufiya government, Egypt. In addition to 7 sputum samples from patients showing signs of tuberculosis as well as blood samples from 92 persons with close contact with animals were also collected.

DNAs were extracted from animal tuberculous lesions and sputum samples from patient to be examined by MTBDR*plus* assay for detection of *Mycobacterium tuberculosis* complex (MTC) and identify strains that show resistance to isoniazid and rifampicin. Moreover, DNAs were also examined for the presence of *M. tuberculosis* by conventional PCR. On the other hand, serum samples from 92 persons in intimate contact with animals were examined by ELISA for the presence of IgG antibodies against *M. tuberculosis*. Of the examined animal samples MTC and *M. tuberculosis* were detected in 88% and 11% respectively, and 29.4% showing drug resistance. All sputum samples from examined patients were *M. tuberculosis* positive, of these 57% showed drug resistance. 5.4% of human serum samples were positive for IgG antibodies against TB. In conclusion, detection of *Mycobacterium tuberculosis* in animals (cattle and buffalo), especially the drug resistant type, is a serious problem to human and animal health and wealth.

Dedication

***My father**, I was hoping to be with me now, you kindly insinuated in my heart the love of knowledge and the patience of the research and now I ask Allah to give you more mercy, forgiveness and put this work, and all who benefit from it in your balance of good deeds.*

*Countless thanks and filial gratitude, which engulf my heart are kept for **my Mother** who continuously encouraged me and give my help and power to pass all difficulties.*

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I dedicate this thesis to my father's soul, my Mother, my husband and my lovely children Malk, Omar and Hana.

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Introduction

1. Introduction

Tuberculosis (TB) remains a major global health problem and infects millions of people each year. TB ranks as the second leading cause of death from an infectious disease worldwide, after the Human Immunodeficiency Virus (HIV) **(WHO,** 2014).

According to World Health Organization (**WHO,** 2015) 10.4 million people fell ill with TB and 1.8 million died from the disease. This report shows higher level for new TB cases and deaths.

Tuberculosis is a disease of poverty, with 90% of the cases occurring in developing countries. The top 22 countries with high TB-burden account for approximately 81.6% of all new TB cases arising each year. Most of these high TB-burden countries are developing countries, which have also experienced the great economic losses due to bovine TB (BTB) in cattle leading to a reduction in animal productivity as well as losses in domestic and international trade in addition to the cost of control and eradication programs **(Chen et al. 2013)**. In the last few decades of *M. tuberculosis* complex, multi-drug resistant (MDR) strains (resistant to at least INH and RIF) have been emerged worldwide, with approximately 480,000 cases as reported by (**WHO,** 2015).

The infection rate of bovine tuberculosis in Egypt decreased from 2009 to 2012 and then increased in 2013. The disease is concentrated in the Egyptian Nile Delta and the Valley more than other sites in the country **(Abdellrazeq et al., 2014)**.

Cattle have historically been considered resistant to *Mycobacterium tuberculosis* **(LoBue *et al.*,(B) 2010).** However some researchers suggest that under certain conditions, human act as source of tuberculosis infection for cattle. The transmission of *Mycobacterium tuberculosis* from humans to animals occurs especially in countries where there is close interaction of humans with the animals **(Ameni et al., 2011)**.

Little attention has been given to transmission of *Mycobacterium tuberculosis* from humans to animals. However, recent studies have reported isolation of *M. tuberculosis* from cattle with prevalence of 4.7%–30.8% in African and Asian countries, respectively (**Prasad *et al.*, 2005, Fetene *et al.*, 2011**), while these records were obtained in countries with the highest incidence of

human tuberculosis. The first reported cases of *M. tuberculosis* in cattle in Western Europe were described in Great Britain and dated back to the 1950s**(Lesslie 1960)**.

The objectives of this study are to:

- Molecular detection of drug resistant *Mycobacterium tuberculosis* complex from tuberculous tissues of cattle and buffaloes through detection of mutations that confer resistance to two most active first-line drugs against TB: Rifampin and Isoniazid.
- Investigate the possible role of cattle and buffalo in the epidemiological cycle of *Mycobacterium tuberculosis*.
- Sero-prevalence of *Mycobacterium tuberculosis* antibodies among human contact with animals.
- This study not only detected MDRTB in humans but also detected this organism in animals as an anthropozoonoses.

Review of literature

2. Review of literature

2.1 History of tuberculosis:

Consumption, phthisis, scrofula, Pott's disease, and the White Plague were all terms used to refer to tuberculosis throughout history.

Pease (1940) reported that TB was documented in Egypt, India, and China as early as 5,000, 3,300, and 2,300 years ago, respectively.

Dubos (1968) reported that Hippocrates (460-375 BC) made the first clinically detailed description of tuberculosis. He called the disease "phthisis". Also, Hippocrates noted that tubercles were found in the tissues of cattle, sheep, and swine.

Sakula (1982) mentioned that Robert Koch may be considered to be the father of the scientific study of tuberculosis.

Beverly *et al.* (1999) stated that in - 1882 – Robert Koch isolated and identified the TB bacillus. Koch established that the disease was spread by exposure to the TB germ. Koch was awarded the Nobel Prize in 1905, and he also made seminal contributions to photomicrography and developed plating and culturing techniques that are still used today. His discovery was followed shortly by the identification of the x-ray by Wilhelm Roentgen in 1895. This allowed early diagnosis and isolation of infected individuals.

Kaufmann and Schaible (2005) mentioned that Clarissimus Galen defined phthisis as an ulceration of the lungs, chest or throat, accompanied by coughs and low fever.

Daniel (2006) presumed that the genus *Mycobacterium* originated more than 150 million years ago.

Schwartz (2009) stated that in the Middle Ages, tuberculosis of the cervical lymph nodes was known as the king’s evil or scrofula.

Recently**, Pfyffer *et al.* (2003)** classified family *Mycobacteriaceae to M.tuberculosis* complex and other mycobacteria.

M. tuberculosis complex includes:-

Mycobacterium tuberculosis: the main cause of tuberculosis in humans.

M. bovis: most commonly found in cattle and other animals such as deer.

M. bovis BCG (BacillusCalmette-Guérin): Still used for vaccine purposes in many parts of the world.

M. africanum: the cause of human tuberculosis in tropical Africa.

M. microti: isolated from rodents and a cause of naturally acquired tuberculosis in guinea pigs, rabbits, llamas, cats, and other warm-blooded animals, and may cause tuberculosis in human.

M. canettii: the youngest member of the *M. tuberculosis* complex.

2.2.Occurrence of *Mycobacterium tuberculosis* in cattle:

Delafosse *et al.* (1995) isolated 39 pathogenic *Mycobacterium* strains (38 *Mycobacterium bovis* and one *Mycobacterium tuberculosis*) from 100 tuberculous tissue samples from slaughterhouse.

Vekemans *et al. (1999)* isolated *M. tuberculosis* from milk and animal tissue samples that raising the question of the transmission of tuberculosis from humans to cattle. They added that tuberculous herdsmen could excrete large numbers of organisms in the late stages of the disease. Sputum, urine, feces and purulent exudate from ruptured lymph node, abscesses or bite wounds can all be implicated in contaminating pasture where cattle graze.

Sulieman and Hamid (2002) isolated 25 *M. bovis*, 21 *M. farcinogenes*, 4 *M. tuberculosis* and one *M. avium* isolates from 120 caseous lesions from slaughtered cattle at slaughterhouses in Sudan.

Pavlik *et al.* (2003) analyzed 11.8 million heads of cattle from six Central European countries (Croatia, the Czech Republic, Hungary, Poland, Slovakia and Slovenia) between years 1990 and 1999. *M. tuberculosis* was isolated only in two countries (Poland and Slovak Republic) from 16 animals (9 cattle, 4 domestic pigs and three wild animals) with tuberculous lesions.

Sreedevi and Krishnappa (2003) found that *M. tuberculosis* in unnatural host may lose their virulence and may not be able to cause serious clinical infection in cattle, but these animals can play an important role in transmitting the disease back to humans.

Singh *et al.* (2004) suggested the possibility of dissemination of a single *M. tuberculosis* strain among animals and humans due to similarity of IS6110 RFLP fingerprints of *M. tuberculosis* strains isolated from animals and human patients.

Mishra *et al.* (2005) from India, recorded that mixed infection from *M. tuberculosis* and *M.bovis* was detected in 22 animals from 64 bovine samples by nested-PCR.

Ocepek *et al.* (2005) found that the isolates from the cattle and farm workers who suffered from pulmonary tuberculosis were the same strains confirmed by molecular typing of isolates by IS6110-based restriction fragment length polymorphism analysis that indicated transmission of *Mycobacterium tuberculosis* from human to cattle.

Pavlik *et al.* (2005) evaluated, during a five year period (2000-2004), the incidence of bovine and human tuberculosis in cattle and other animals from six Central European countries. *M. tuberculosis* was diagnosed in four cattle and two pigs in Poland and in one cattle in Slovenia.

Prasad *et al.* (2005) investigated extra-pulmonary clinical samples obtained from cattle and humans and identified mixed infection (*M. tuberculosis* and *M.bovis*) in cattle at rate 35.7 % and 8.7% in humans. The detection of mixed infection with the mycobacterial pathogenic in humans and bovines denotes the prospect of potential transmission of these pathogens from humans to cattle (zoonosis) and vice versa (reverse zoonosis).

Regassa *et al.* (2008*)* reported two (18%) *Mycobacterium tuberculosis* isolates from 11 cattle having active tuberculosis and owned by farmers with active tuberculosis. They added that cattle owned by farmers with active tuberculosis were four times more likely to have tuberculosis than cattle owned by farmers with no active tuberculosis.

Berg *et al.* (2009) from Ethiopia, suggested human to animal transmission of *M. tuberculosis* due to isolation of *M. tuberculosis* from eight cattle from 32,800 inspected cattle at slaughterhouses.

Chen *et al*. (2009) from China**,** identified six *M. tuberculosis* isolates using multiplex PCR from thirty-eight cows in a herd determined to be positive for bovine tuberculosis. *M. bovis* and *M. tuberculosis* infection induced comparable pathology in cattle in both gross pathology and

histopathology based on the qualitative assessment of the sampled lung tissues. The spoligo-typing demonstrated that cow

M. tuberculosis isolates belonged to Beijing family strains.

YueQin *et al.* (2010) applied compound (multiplex) PCR for the rapid identification of *Mycobacterium* characteristic maker genes (MTP40 gene, alpha -antigen gene and IS6110), to test the specimens from human and bovine TB cases. The specimens from human TB cases were positive for *Mycobacterium tuberculosis*, and the specimens from bovine TB cases were positive for *M. bovis* and *M. tuberculosis*. They concluded that dairy cattle TB cases are not only the possible source of human infection with *M. bovis*, but also a possible source of human infection with *M. tuberculosis*.

Ameni *et al.* (2011) collected samples from tuberculous cattle from two different management systems in central Ethiopia. 27% of isolates from grazing cattle were *M. tuberculosis*. The practice of local farmers discharging chewed tobacco directly into the mouths of pastured cattle was identified as a potential route of human-to-cattle transmission of *M. tuberculosis*.

Fetene *et al.* (2011) collected samples from two hundred and ten cattle owners and 1220 heads of their cattle. They isolated *M. tuberculosis* (15.4%) from milk collected from tuberculin-positive cows and (74.5%) from sputum and fine needle aspiration specimens of tuberculosis patient cattle owners. They stated that the prevalence of tuberculosis was significantly higher in cattle owned by tuberculosis patients than in cattle owned by non-tuberculosis owners, and infection with tuberculosis was threefold greater in cattle owned by tuberculosis-positive owners.

Jenkins *et al.* (2011) from Nigeria recorded that from years 2005 to 2007, *Mycobacterium tuberculosis* complex strains were isolated from cattle, goats and pigs. Samples were collected at the Bodija abattoir and from tuberculosis patients and livestock traders in Ibadan, Southwestern Nigeria. Seventy four isolates obtained from humans (24) and livestock (50) were identified as MTC strains. Thirty two isolates were spoligo-typed. Nineteen of these 32 isolates were identified as *M. tuberculosis* whilst 13 were identified as *M. bovis. M. bovis* was isolated from two humans, whereas *M. tuberculosis* was isolated from a bovine, a pig and a goat.

Romero *et al.* (2011) reported that during 2007–2009 three cases of tuberculosis caused by *M. tuberculosis* were detected in 3 unrelated cattle farms, 2 of them free from tuberculosis. In all cases, staff of the farms had active tuberculosis. The human strain