# Tuberculosis in Nonhuman Primates - the disease

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## 1.  Introduction

Simian tuberculosis is one of the most important bacterial diseases of nonhuman primates because of its ubiquitous and insidious nature and its ability to spread rapidly. Outbreaks of tuberculosis have been reported in nonhuman primate colonies almost as long as primates have been used as experimental animals or kept in zoological gardens. The tuberculous monkey is a health hazard especially to other monkeys in the group, but (re)transmission of the infection to humans has been reported as well. Significant progress has been made in reducing the incidence of tuberculosis in captive nonhuman primates. In spite of reasonable precautions, outbreaks continue to occur and tuberculosis remains a serious threat to the health of nonhuman primates and their caretakers. Outbreaks have severe economic consequences due to animal losses, disruption of research and costs related to disease control. Therefore, persons working with nonhuman primates should be familiar with the disease and preventive measurements.

## 2.  The agent

Tuberculosis and mycobacteriosis are fatal diseases of New and Old World monkeys causing high morbidity and mortality (Bennett et al., 1998; Brack, 1987). Simian tuberculosis is a disease most often contracted from humans. Most of the clinical cases are induced by *Mycobacterium (M.) tuberculosis*, but the incidence of *M. bovis* infections is increasing (Keet et al., 1996; Stetter et al., 1995; Thorel et al., 1998; Zumpe et al., 1980). Infections with other members of the mycobacterium tuberculosis complex (*M. africanum*, *M. microti*, *M. canetti*) are rarely reported (Thorel, 1980). Mycobacteria other than tuberculosis (MOTT) may lead to a tuberculosis like disease in nonhuman primates, which is less infective. In most cases, mycobacteriosis is induced by *M. avium-intracellulare* complex, which is composed of more than 30 serotypes. These bacteria are non-pathogenic, saphrophytic, non-tuberculous mycobacteria belonging to Runyon group III. Agents

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of the *M. avium-intracellulare* complex have been frequently isolated from macaque species with atypical mycobacterial infection causing opportunistic infections in immunodeficient animals associated with simian immunodeficiency virus (SIV) (Mansfield and Lackner, 1997). Other seldom occurring mycobacteria species include *M. kansasii, M. simiae*, and *M. chelonei* (Didier et al., 1999; Parsons et al., 2010). There are only slight differences in the clinical course of the disease, and the mycobacterial species can only be differentiated by culture or by PCR. In general mycobacteria are acid fast, rod-shaped facultative intracellular bacilli that grow on artificial media like Lowenstein-Jensen or Middlebrook and Cohn media at 37°C. They grow slowly and form flower-shaped colonies.

## 3. Pathogenesis

The main route of infection is via the respiratory tract by inhalation of fine particles containing the bacilli. Transmission may occur by ingestion, especially in cases of *M. bovis* infection after feeding contaminated meat. Other ways of transmission are through fight wounds, tattoo machines, needles, rectal thermometers, nets and gloves. Although the disease usually affects the respiratory tract, tuberculosis can disseminate to almost any organ and it should therefore be regarded as a systemic infection.

Disease pathogenesis involves phagocytosis of organisms by mononuclear phagocytes including tissue-resident macrophages and dendritic cells (DC). These cells may vary in their capacity to kill the organisms, leading to diverse possibilities for infection outcome and lesion morphology. Successful intracellular killing of mycobacteria enables processing of the agent and antigen presentation for immune response by T-lymphocytes. T-cells, as well as peripheral blood monocytes, are recruited to the infection site by various macrophage-derived cytokines. Further activation of mononuclear phagocytes occurs in these sites in response to mycobacterial products, and cytokines released by the activated lymphocytes. Activated macrophages phagocyte the bacteria and tend to transform into immobile epitheloid cells forming epitheloid granulomas. Non-resident macrophages and particularly DC may move to regional lymph nodes and other tissues, where, if intracellular killing has not been completed, the infection progresses and associated inflammatory response continues. Additional processes that contribute to the classical morphology of tuberculous lesions include the fusion of macrophages to

form multinucleated Langhans-type giant cells (Hines et al., 1995).

## 4. Clinical symptoms

Tuberculosis occurs in prosimians, New and Old World monkeys and apes. Although some species may be more or less susceptible to the disease, all nonhuman primates can develop tuberculosis. Old World monkeys are considerably more sensitive than apes and New World monkeys (Brack, 1987). The severest outbreaks were observed in macaque species and African green monkeys. Based on the incidence, the disease is common in Asian monkey species, especially those from India, less frequent in African species and rare in New World monkeys and prosimians. In New World monkeys, which generally are regarded as highly resistant to the disease, most of the documented cases occurred in cebids (Brack, 1992). After primary infection, a spectrum of clinical outcomes is possible. A fulminant rapid progression of the disease is commonly seen in macaques. Chronic debilitating disease courses are possible as well as latent infections without overt disease are documented depending on the infective dose. In general, the clinical symptoms are minimal or absent until the disease is far advanced (Ialeggio, 1997). Frequently, primates are found dead with no previous clinical history. If clinical symptoms are present, the main symptoms are unspecific and include weakness, paralysis, loss of appetite, loss of weight, dull hair coat, coughing, and general depression. Intermittent coughing is a characteristic clinical sign for pulmonary tuberculosis. Symptoms of extrapulmonary tuberculosis are determined by the involved organs. Cases of primary and secondary cutaneous tuberculosis are characterized by non-healing wounds, draining ulcers or fistulous tracts combined with enlargement of lymph nodes. Vertebral tuberculosis results in paraplegia or kyphosis, and cerebral tuberculosis causes epileptiform seizures. Intestinal tuberculosis leads to severe diarrhea. Tuberculous animals develop a normocytic, normochromatic anemia (Bennett et al., 1998).

In rhesus monkeys, tuberculosis normally manifests as pulmonary lesions. The course of disease may vary depending on the macaque species involved. Rhesus monkeys may develop an acute and progressive form of tuberculosis (Langermans et al., 2001). Cynomolgus monkeys appear to be more resistant and are able to carry the infection as a subclinical form mimicking latent infection in humans (Capuano et

al., 2003; Langermans et al., 2001; Walsh et al., 1996). **In general, intermittent coughing and chronic weight loss should always be regarded as possible indicators for the disease.**

## 5. Pathology

The lesions of primary tuberculosis in nonhuman primates can vary from non-detectable lesions to widely disseminated, firm yellowish-white or greyish nodules ranging in size from pin point dimensions to several millimeters in diameter (Fig. 1, 2). Enlarged tracheobronchial lymph nodes with caseous necrotic centers are the most prominent lesions (Fig. 1, 3). Palpable firm nodules may affect all major organs, but the lung is the most commonly affected organ system. Gross lesions also include large cavitary and coalescing lesions within the lung and tubercles extending into the thoracic pleura. Advanced stages of the disease are characterized by secondary spread to liver, spleen, kidney and various lymph nodes (Fig. 5, 6) (Garcia et al., 2004; King, 1993).

Tuberculous nodules are found less frequently within the cerebrum, omentum, uterus and ovary, peripheral lymph nodes, skin, and mammary gland. Tuberculous lesions within the vertebrae and adjacent spinal cord similar to Pott`s disease in humans have also been described in nonhuman primates.

Microscopic findings vary depending upon the duration and extension of the disease. Early stages of the disease are characterized by small granulomas consisting of circumscribed accumulations of epitheloid cells and few Langhans-type giant cells confined to the lung or the intestinal tract. Acid fast bacilli are only sporadically found within the lesions. Advanced stages of the disease are characterized by the classic tubercle formation. Tubercles are typical granulomas of varying size containing a caseous center consisting of acellular necrotic debris. The central cores are surrounded by a zone of epitheloid cells interspersed with only a few Langhans-type giant cells. In contrast to other animal species, a fibrous capsule is usually not found in nonhuman primates. Calcification is usually rare or lacking. Acid fast stains, such as Ziehl-Neelsen and Fite-Faraco-Kinyoun can be used to demonstrate acid fast bacilli in the epitheloid or multinucleated giant cells or even extracellularly. The number of acid fast bacilli can vary considerably. The tuberculous lesions are

normally accompanied by similar alterations of the regional lymph nodes.

Differential diagnoses of microscopic lesions seen with tuberculosis are other granulomatous diseases induced by foreign bodies (e.g. kaolin) or by mycotic, protozoan and parasitic organisms (King, 1993; Malaga et al., 2004).

## 6. Treatment

Treatment of tuberculous monkeys and apes has been undertaken and was successful in some cases. Multidrug treatment may be considered, but only if appropriate isolation and containment facilities can be provided and accurate detection of infection is possible. Biosafety level 3 conditions would be necessary for husbandry of animals under therapy. Treatment in nonhuman primates includes the use of a multiple drug regimen with isoniazid, rifampicin and ethambutol, or streptomycin and isoniazid over a period of at least 9 to 12 months, sometimes even up to 30 months. Treatment must be carried out in conjunction with culture, clinical examination and ultrasound.

Single drug therapy with isoniazid as a preventive or as sole therapeutic agent has been proven to be ineffective. The use of isoniazid has the potential drawbacks of rendering tuberculin testing inaccurate, causing behavioural changes as well as inducing pyridoxine deficiency.

Multidrug chemotherapy in tuberculosis using two or more drugs has been effective in macaques and apes. The use of multiple agents decreases the possibility of developing antibiotic resistance and allows grouping of agents that attack the mycobacteria at different stages of their life cycle. Effective drug combinations include isoniazid and streptomycin, isoniazid and p-aminosalicyclic acid or isoniazid, ethambutol, and rifampicin (Indzhila et al., 1977; Ward et al., 1985; Wolf et al., 1988). In all cases of successful treatment, antibiotic sensitivity of the isolated organism had been determined beforehand. Considering costs and ethical issues involved with containment of the disease, treatment is only advisable for very valuable animals.

## 7. Summary

Tuberculosis of nonhuman primates is a disease most often acquired after transmission from humans and then spreading within nonhuman primate populations. The incidence of human tuberculosis is still increasing. Therefore thoroughly monitoring of people working with nonhuman primates is mandatory. The disease is a

significant anthropozoonosis, and care must be taken in handling animals suspected of carrying the disease. Infection occurs by inhalation or occasionally by digestion. The disease progresses slowly, and clinical signs may be absent until the disease has become advanced. The most common sign is coughing which should be regarded as an indicator for the disease.

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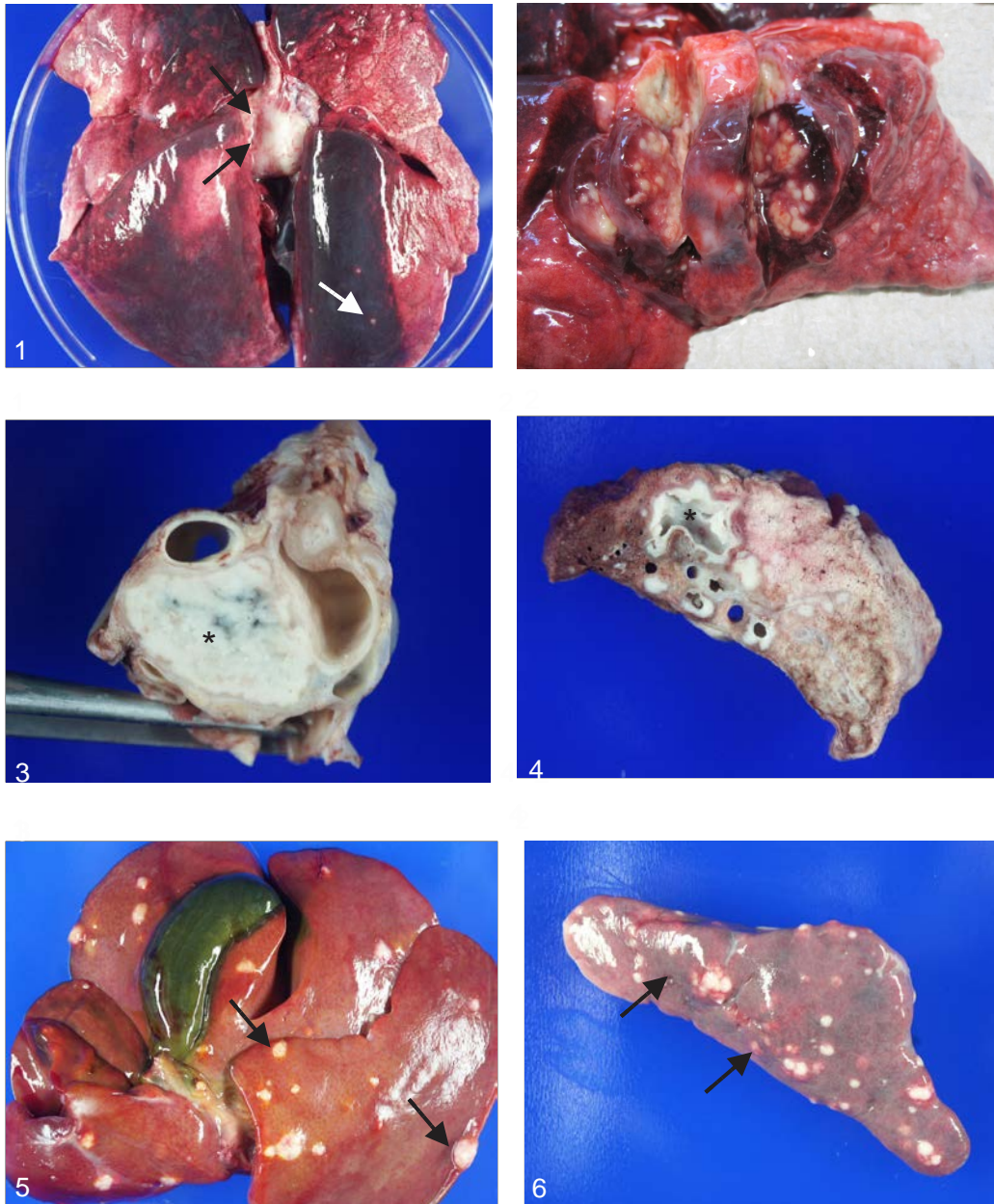
## 9. Figures



Figure legends:

Fig.1. Lung; rhesus monkey, case G 8607. Enlargement of the bronchopulmonal lymph nodes surrounding the bifurcation of the trachea (black arrow) and small pin point shaped granulomas distributed throughout the parenchyma of the right caudal lung lobe (white arrow).

Fig. 2. Lung; rhesus monkey, case G 8609. Severe granulomatous pneumonia with large confluent granulomas within a cranial lung lobe.

Fig. 3. Lymph node; rhesus monkey, case G 8606. Enlarged bronchopulmonal lymph node with caseous necrotic center (asterix).

Fig. 4. Lung; rhesus monkey, case G 8606. Multiple granulomas of different size within the lung parenchyma developing cavitary and coalescing lesions (asterix).

Fig. 5. Liver; rhesus monkey, case G 8591. Multiple granulomas of different size distributed diffusely within the liver parenchyma indicating systemic spread of the disease.

Fig. 6. Spleen; rhesus monkey, case G 8591. Multiple granulomas of different size distributed diffusely within the spleen parenchyma indicating systemic spread of the disease.