**UFRRJ**
**INSTITUTO DE VETERINÁRIA**
**PROGRAMA DE PÓS-GRADUAÇÃO EM MEDICINA VETERINÁRIA**
**PATOLOGIA E CIÊNCIAS CLÍNICAS**

**DISSERTAÇÃO**

# TUBERCULOSE EM PRIMATAS NÃO HUMANOS DO NOVO E DO VELHO MUNDO

**Asheley Henrique Barbosa Pereira**

**2021**

**UNIVERSIDADE FEDERAL RURAL DO RIO DE JANEIRO**
**INSTITUTO DE VETERINÁRIA**
**PROGRAMA DE PÓS-GRADUAÇÃO EM MEDICINA VETERINÁRIA**
**PATOLOGIA E CIÊNCIAS CLÍNICAS**

# TUBERCULOSE EM PRIMATAS NÃO HUMANOS DO NOVO E VELHO MUNDO

**ASHELEY HENRIQUE BARBOSA PEREIRA**

*Sob a Orientação do Professor*
**Daniel Guimarães Ubiali**

Dissertação submetida como requisito parcial para obtenção do grau de **Mestre**, no Programa de Pós-Graduação em Medicina Veterinária, Área de Concentração Patologia Animal

Seropédica, RJ
Novembro de 2021

Universidade Federal Rural do Rio de Janeiro
Biblioteca Central / Seção de Processamento Técnico

Ficha catalográfica elaborada
com os dados fornecidos pelo(a) autor(a)

P436t

Pereira, Asheley Henrique Barbosa, 1997-
Tuberculose em primatas não humanos do Novo e Velho mundo / Asheley Henrique Barbosa Pereira. - Seropédica, 2021.
66 f.: il.

Orientador: Daniel Guimarães Ubiali.
Dissertação(Mestrado). -- Universidade Federal Rural do Rio de Janeiro, Programa de Pós-graduação em Medicina Veterinária, 2021.

1. Histopatologia. 2. Spillover. 3. Patologia Veterinária. 4. Tuberculose. 5. Zoonose. I. Ubiali, Daniel Guimarães , 1985-, orient. II Universidade Federal Rural do Rio de Janeiro. Programa de Pós graduação em Medicina Veterinária III. Título.

**UNIVERSIDADE FEDERAL RURAL DO RIO DE JANEIRO**
**INSTITUTO DE VETERINÁRIA**
**PROGRAMA DE PÓS-GRADUAÇÃO EM MEDICINA VETERINÁRIA**
**PATOLOGIA E CIÊNCIAS CLÍNICAS**

**ASHELEY HENRIQUE BARBOSA PEREIRA**

Dissertação submetida como requisito parcial para obtenção do grau de **Mestre em Medicina Veterinária**, no Programa de Pós-Graduação em Medicina Veterinária, Área de Concentração Patologia Animal

DISSERTAÇÃO APROVADA EM: 08/11/2021

_____
Daniel Guimarães Ubiali (Dr.) UFRRJ
(Orientador)

_____
Alcides Pissinatti (Dr.) INEA CPRJ

_____
Ricardo Barbosa de Lucena (Dr.) UFPB

_____
Andressa Ferreira da Silva Spyrides (Dra.) UFRRJ

***ATA DE DEFESA DE TESE Nº 159/2024 - PPGMV (12.28.01.00.00.00.00.51)***

***(Nº do Protocolo: NÃO PROTOCOLADO)***

***(Assinado digitalmente em 06/07/2024 14:01 )***
*ANDRESSA FERREIRA DA SILVA SPYRIDES*
*PROFESSOR DO MAGISTERIO SUPERIOR*
*DeptMCV (12.28.01.00.00.00.00.53)*
*Matrícula: ###327#6*

***(Assinado digitalmente em 05/07/2024 16:13 )***
*DANIEL GUIMARAES UBIALI*
*PROFESSOR DO MAGISTERIO SUPERIOR*
*DESP (12.28.01.00.00.00.00.52)*
*Matrícula: ###017#8*

***(Assinado digitalmente em 08/07/2024 09:12 )***
*ASHELEY HENRIQUE BARBOSA PEREIRA*
*DISCENTE*
*Matrícula: 2020#######8*

***(Assinado digitalmente em 07/07/2024 11:08 )***
*RICARDO BABROSA DE LUCENA*
*ASSINANTE EXTERNO*
*CPF: ###.###.523-##*

***(Assinado digitalmente em 10/07/2024 16:13 )***
*ALCIDES PISSINATTI*
*ASSINANTE EXTERNO*
*CPF: ###.###.317-##*

*Visualize o documento original em https://sipac.ufrrj.br/documentos/ informando seu número:* ***159****, ano:* ***2024****, tipo:* ***ATA DE DEFESA DE TESE****, data de emissão:* ***05/07/2024*** *e o código de verificação:* ***e530998d4b***

## AGRADECIMENTOS

À minha família, que é a base de tudo. Agradeço aos meus pais e irmãos e todos os demais que fizeram o possível e o impossível para auxiliar e garantir a minha permanência no ambiente acadêmico. Amo vocês e serei eternamente grato por tê-los na minha trajetória.

Ao meu orientador Daniel Ubiali por todos ensinamentos tanto acadêmicos quanto pessoais. A minha família de Seropédica composta por poucos amigos e, principalmente, pelo meu orientador Daniel Ubiali, sua companheira Bianca Weiss e minha irmã mais nova, a Lis por terem me acolhido e proporcionado a minha vida mais leve.

À Marvin e as meninas, Pandora e Molly por dividirem moradia e proporcionarem ótimos momentos. Aos meus amigos e companheiros do Setor de Anatomia Patológica, em especial à Nathalia Carvalho, Fernanda Freitas, Gabriela Pereira, Karla Nascimento, Luís Tondo, Carolina Melo e Marina Souza. Obrigado por tudo.

À médica veterinária de animais selvagens Bruna Emely por todos os ensinamentos e pela confiança. A equipe do Centro de Primatologia do Rio de Janeiro e do Instituto de Ciência e Tecnologia em Biomodelos da Fiocruz, em especial a Cláudia Lopes.

Aos professores de patologia, microbiologia e biologia molecular da Universidade Federal de Mato Grosso. Meus sinceros agradecimentos ao professor Edson Moleta, a professora Valéria Dutra e professor Luciano Nakazato pelo apoio e parceria.

O presente trabalho foi realizado com o apoio da Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) – Código de Financiamento 001.

## RESUMO

PEREIRA, Asheley Henrique Barbosa. **Tuberculose em primatas não humanos do Novo e Velho Mundo**. Seropédica, RJ. 2021. 66p. Dissertação (Mestrado em Medicina Veterinária, Patologia Animal). Instituto de Veterinária, Programa de Pós-Graduação em Medicina Veterinária – Patologia e Ciências Clínicas, Universidade Federal Rural do Rio de Janeiro, Seropédica, RJ, 2021.

A tuberculose é uma doença infecciosa crônica que causa alta morbidade e mortalidade em humanos e primatas não humanos, especialmente em cativeiro. No Brasil, a incidência desta doença em humanos tem aumentado lentamente nos últimos anos, o que a tornou uma doença classificada como reemergente. A tuberculose tem distribuição cosmopolita e é causada pelos microrganismos pertencentes ao complexo *Mycobacterium tuberculosis*. Neste estudo são descritos os achados epidemiológicos, clínicos, patológicos e moleculares dos diferentes estágios da tuberculose em primatas do Novo e do Velho Mundo naturalmente infectados com o Complexo *Mycobacterium tuberculosis* e mantidos sob cuidados humanos no Rio de Janeiro, Brasil. Quinze primatas não humanos de cinco colônias diferentes foram incorporados ao estudo, dos quais 60% (9/15) foram primatas do Velho Mundo e 40% (6/15) primatas do Novo Mundo. Os estágios da tuberculose foram classificados de acordo com os achados macroscópicos e histopatológicos em: tuberculose crônica-ativa, tuberculose extrapulmonar, estágio de ativação-inicial da tuberculose e reativação latente. Entre os primatas do Velho Mundo, 66,7% (6/9), todos macacos rhesus (*Macaca mulatta*), apresentaram pneumonia granulomatosa multifocal severa. Em todos os casos de tuberculose em primatas do Velho Mundo, granulomas típicos foram observados em pelo menos um órgão, independentemente do estágio da doença. Nos primatas do Novo Mundo, os granulomas pulmonares típicos foram vistos em 16,7% (1/6) dos casos, apenas no estágio de reativação latente em um espécime de Cuxiú (*Chiropotes utahickae*). Neste estudo, 66,7% (6/9) dos primatas do Velho Mundo e 83,3% (5/6) dos primatas do Novo Mundo apresentaram alterações pulmonares na avaliação histológica. O diagnóstico de tuberculose nos primatas não humanos neste estudo foi baseado nos achados patológicos, imuno-histoquímicos, moleculares e bacteriológicos. Embora a apresentação típica tenha sido observada em alguns casos, a ausência de granuloma pulmonar não excluiu a ocorrência de tuberculose em primatas não humanos do Novo e do Velho Mundo. A tuberculose deve ser incluída como causa de pneumonia intersticial com macrófagos espumosos nos primatas não humanos do Novo Mundo. Devido à alta sensibilidade da imuno-histoquímica com anti-*Mycobacterium tuberculosis*, sugerimos a incorporação dessa técnica como ferramenta diagnóstica da tuberculose em primatas não humanos, mesmo quando as alterações típicas não são observadas.



**Palavras-chave:** *Spillover*, Tuberculose, Zoonose.

## ABSTRACT

PEREIRA, Asheley Henrique Barbosa. **Tuberculosis in nonhuman primates of the New and the Old World**. Seropédica, RJ. 2021. 66p. Dissertation (Master in Veterinary Medicine, Animal Pathology). Postgraduate Program in Veterinary Medicine – Pathology and Clinical Sciences, Federal Rural University of Rio de Janeiro, Seropédica, RJ, 2021.

Tuberculosis is a chronic infectious disease that causes high morbidity and mortality in humans and non-human primates, especially in captivity. In Brazil, the incidence of this disease in humans has slowly increased in recent years. Tuberculosis has a cosmopolitan distribution and is caused by microorganisms belonging to the Mycobacterium tuberculosis complex. This study describes the epidemiological, clinical, pathological and molecular findings of the different stages of tuberculosis in New and Old World primates naturally infected with the *Mycobacterium tuberculosis* Complex and kept under human care in Rio de Janeiro, Brazil. Fifteen nonhuman primates from five different colonies were incorporated into the study. There are 60% (9/15) Old World Monkeys and 40% (6/15) New World Monkeys. According to the gross and histopathologic findings, the lesions in nonhuman primates of this study are classified into the chronic-active, extrapulmonary, early-activation or latent-reactivation tuberculosis stage. Among the Old World Monkey, 66.7% (6/9) of nonhuman primates, all rhesus monkeys (*Macaca mulatta*), showed severe granulomatous pneumonia. In all Old World Monkeys cases, typical granulomas were seen in at least one organ regardless of the stage of the disease. In the New World Monkeys, the typical pulmonary granulomas were seen in 16.7% (1/6) of the cases, just in the latent-reactivation stage in Uta Hick's Bearded Saki (*Chiropotes utahickae*). In this study, 66.7% (6/9) of Old World Monkeys (OWM) and 83.3% (5/6) of New World Monkeys (NWM) showed pulmonary changes at the histological evaluation. The tuberculosis diagnosis in the nonhuman primates in this study was based on pathological, immunohistochemical, molecular, and bacteriological culture. Although the typical presentation was observed in some cases, the absence of pulmonary granuloma did not exclude the tuberculosis occurrence in nonhuman primates of the Old and New World. Tuberculosis should be included as a cause of interstitial pneumonia with foamy macrophages infiltration in the New World nonhuman primates. Due to the high sensitivity of immunohistochemistry with anti-*Mycobacterium tuberculosis*, we suggest the addition of this technique as a diagnostic tool of tuberculosis in the nonhuman primates even when the typical changes are not seen.



**Keywords:** *Spillover*, Tuberculosis, Zoonosis.

## LISTA DE ABREVIAÇÕES E SÍMBOLOS

| | |
|---|---|
| TB | Tuberculose |
| PNHs | Primatas não humanos |
| CMTB | Complexo *Mycobacterium tuberculosis* |
| *Mtb* | *Mycobacterium tuberculosis* |
| *Mbo* | *Mycobacterium bovis* |
| PVM | Primatas do Velho Mundo |
| PNM | Primatas do Novo Mundo |
| PCR | Reação em cadeia pela polimerase |
| IFN-γ | Interferon Gama |
| IHQ | Imuno-histoquímica |

## SUMÁRIO

# 1 INTRODUÇÃO

A tuberculose (TB) é uma doença infecciosa crônica que causa alta morbidade e mortalidade em humanos e primatas não humanos (PNHs), especialmente em primatas de cativeiro (WARIT et al., 2020). A TB tem distribuição cosmopolita e é causada pelos microrganismos pertencentes à família Mycobacteriaceae e ao complexo *Mycobacterium tuberculosis* (DOOD et al., 2021). O complexo *Mycobacterium tuberculosis* (CMTB) refere-se a um grupo de espécies de micobactérias estreitamente relacionadas. De modo geral, *M. tuberculosis* (*Mtb*) e *M. africanum* são os principais agentes etiológicos da TB em humanos (KANABALAN et al., 2021).

As espécies que causam TB pertencem ao gênero *Mycobacterium* e são altamente adaptadas aos hospedeiros e capazes de infectar diferentes espécies de mamíferos. O principal agente etiológico da TB em PNHs é o *Mtb* (MANSFIELD; FOX, 2019), embora *M. bovis* (*Mbo*), *M. africanum* e *M. microtii* também sejam relatados (HENRICH et al., 2007; LIN et al., 2008; PARSONS et al., 2009; VIA et al., 2013). Adicionalmente, *Mtb* e *M. africanum* são causas frequentes de TB em humanos, *Mbo* em bovinos, *M. caprae* em ovinos e caprinos, *M. pinipedii* em leões marinhos e focas, *M. microti* em ratos e *M. orygis* em órixes (SMITH et al., 2006; INGEN et al., 2012).

Algumas espécies de PNHs desenvolvem TB idêntica à observada em humanos (CAPUANO et al., 2003) e são potencialmente susceptíveis à infecção por *Mtb* (GOOD 1984). Devido à essa semelhança, os PNHs são considerados o padrão ouro para modelagem da infecção por *Mtb* (FOREMAN et al., 2017). Os PNHs pertencem à ordem Primatas, que é dividida em duas semi-ordens: Strepsirrhini (lêmures e lorises) e Haplorhini, (antropoides e társios). Os táxons pertencentes à Haplorhini, na subordem Antropoidea, são divididos em duas parvo-ordens: os Catarrhini (Primatas do Velho Mundo) encontrados na África e Ásia e os Platyrrhini (Primatas do Novo Mundo) que ocorrem exclusivamente no continente americano (FLEAGLE; SEIFFERT, 2020).

De modo geral, os Primatas do Velho Mundo (PVM) são mais susceptíveis à TB em comparação aos Primatas do Novo Mundo (PNM) (BRACK, 1987; MONTALI et al., 2001). Embora a TB em PVM seja bem conhecida e bastante relatada, a condição é considerada incomum em PNM (MÄTZ-RENSING; LOWENSTINE, 2018). As espécies de PVM mais sensíveis à TB são os macacos rhesus (*Macaca mulatta*) e os macacos-rabo-de-porco (*M. nemestrina*) (GHODBANE; DRANCOURT, 2013). O macaco rhesus (*M. mulatta*) e o cinomolgus (*M. fascicularis*) são as principais espécies submetidas aos bioensaios e à infecção experimental por *Mtb* (SCANGA; FLYNN 2014; FOREMAN et al., 2017).

A ocorrência de TB em PNHs de vida livre é incomum, no entanto, é uma das principais doenças infecciosas diagnosticadas em cativeiro (HINES et al., 1995; ROCHA et al., 2011), o que a torna uma potencial ameaça para as colônias de PNHs (WALSH et al., 1996). A principal rota de infecção é a via aerógena, através da inalação de micropartículas que contém o bacilo infectante (SAKAMOTO, 2012). O contato próximo com humanos favorece a ocorrência da doença em PNHs cativos (MÄTZ-RENSING et al., 2015). Outras fontes alternativas de infecção relatadas em PNHs são a incorporação de primatas recém importados à colônia (SHIPLEY et al., 2008), contato ou agressões diretas com outros animais infectados (GHODBANE; DRANCOURT, 2013), tatuagem (ALLEN; KIARD, 1958) e utilização de fômites infectados como termômetros (RIORDAN, 1943).

PNHs com TB, seja por infecção experimental ou natural, desenvolvem sinais clínicos indistinguíveis da TB humana (GHODBANE; DRANCOURT 2013). Observa-se nestes casos, um amplo espectro de manifestações clínicas, incluindo os casos de infecção latente, tuberculose ativa, tuberculose pós-primária ou extrapulmonar e ainda, manifestações rapidamente progressivas em decorrência à reativação da tuberculose (LIN et al., 2009).

Frequentemente os PNHs com TB apresentam anorexia, perda de peso, letargia e tosse (GHODBANE; DRANCOURT 2013).

No Brasil, há poucos estudos sobre a ocorrência de TB em PNHs e, de modo geral a literatura se restringe a relatos de casos isolados (ROCHA et al., 2011; EHLERS et al., 2020). Nesse contexto, faz-se necessário a realização de estudos detalhados e descritivos sobre a TB em PNHs a fim de contribuir com as normas técnicas, programas e planos nacionais de criação e conservação dos PNHs de cativeiro e de vida livre. O objetivo deste estudo é descrever os aspectos epidemiológicos, clínicos, patológicos e moleculares de Primatas Não Humanos do Novo e do Velho Mundo diagnosticados com tuberculose, provenientes de cativeiro no Estado do Rio de Janeiro, Brasil.

# 2 REVISÃO DE LITERATURA

## 2.1 Agente Etiológico

O Complexo *Mycobacterium tuberculosis* (CMTB) compreende um grupo de micobactérias intimamente relacionadas geneticamente e incluem as espécies *M. tuberculosis*, *M. africanum*, *M. bovis*, *M. canettii*, *M. microti*, *M. pinnipedii* e *M. caprae* (FORRELLAD et al., 2013). Adicionalmente, duas espécies foram recentemente incorporadas ao CMTB e incluem *M. orygis* e *M. mungi* (INGEN et al., 2012; PYFFER, 2015).

As micobactérias são bacilos álcool-ácido resistentes, não móveis, não esporulantes, ligeiramente gram-positivos que aparecem microscopicamente como bastonetes retos ou ligeiramente curvos, com 1 a 4 µm de comprimento e 0,3 a 0,6 µm de largura (SAKAMOTO 2012). Os microrganismos do CMTB são bacilos intracelulares facultativos, aeróbicos, embora algumas possam viver em baixa tensão de oxigênio.

Os membros do gênero *Mycobacterium* expressam ácidos micólicos únicos no envelope celular que desempenham um papel crítico na estrutura e função da parede celular (BARRY et al, 1998). De acordo com FORRELAND et al., (2013) os fatores de virulência de *Mycobacterium* spp. podem ser divididos com base na sua função, características moleculares ou localização celular. Esses fatores incluem o metabolismo de lipídios e ácidos graxos, presença de proteínas do envelope celular, incluindo as que inibem efetores antimicrobianos do macrófago, proteínas quinases, proteases como as metaloproteases, entre outras proteínas com função ainda desconhecida.

## 2.2 Epidemiologia

### 2.2.1 Primatas não humanos

Embora determinadas espécies possam ser mais ou menos suscetíveis, todos os primatas não humanos (PNHs) podem desenvolver tuberculose (TB) (SIMMONS; GIBSON 2012). Os surtos mais graves de tuberculose (TB) são relatados entre os PVM, enquanto os casos em PNM são pouco comuns (Brack, 1987). Existem grandes diferenças na suscetibilidade à TB entre as espécies de PNHs (MÄTZ-RENSING; LOWENSTINE, 2018). As razões para esta disparidade não são claras, embora acredita-se que essa diferença se deva à processos evolutivos entre o hospedeiro e o patógeno (MANSFIELD; FOX, 2019). Entre os membros do CMTB, as principais espécies que causam TB em PNHs são *Mtb* e *Mbo* e podem causar doença esporádica ou epizoótica de alta morbidade e mortalidade em colônias de cativeiro (MANSFIELD; FOX, 2019).

A incidência de TB em primatas do Velho Mundo (PVM) é alta, com alta prevalência em macacos rhesus e tende a ser uma doença rapidamente progressiva, caracterizada por disseminação sistêmica (MÄTZ-RENSING et al., 2015). Os saguis (*Callithrix* spp.), macacos-prego (*Sapajus* spp.), macacos-aranha (*Ateles* spp.) e os macacos-de-cheiro (*Saimiri* spp.) parecem ser os gêneros mais suscetíveis à TB entre os PNM, enquanto o gênero *Aotus* apresenta certo grau de resistência para a doença (BONE; SOAVE, 1970; MORELAND, 1970; LEATHER; HAMM, 1976; FORTMAN et al, 2001; OBALDÍA et al., 2018). Em um estudo recente realizado em uma colônia no Rio Grande do Sul, EHLERS et al. (2020) relataram que a TB pode causar uma doença altamente contagiosa e progressiva com alta mortalidade em macacos-pregos-pretos (*Sapajus nigritus*).

A TB raramente acomete PNHs de vida livre, embora exista um relato de detecção de *Mtb* em uma população selvagem de babuínos em ambientes periurbanos na África do Sul (PARSONS et al., 2009). O contato com o homem infectado é a principal causa de exposição ao *Mtb* para esses animais. Uma vez estabelecida em uma colônia, a doença se espalha rapidamente para animais alojados levando a uma epizootia com alta morbidade e mortalidade (SIMMONS; GIBSON 2012). A tuberculose (TB) em primatas do Velho Mundo (PVM) comumente resulta em uma doença debilitante e progressiva. A latência não é considerada uma característica comum, mas os macacos cynomolgus (*M. fascicularis*) podem desenvolver um curso de doença clinicamente mais silencioso (LIN et al., 2009). A ocorrência de TB em primatas não humanos (PNHs) de cativeiro tem consequências econômicas significativas devido à morte e custos relacionados ao diagnóstico e controle da doença (MÄTZ-RENSING et al., 2015).

### 2.2.2 Humanos

A TB é considerada a doença bacteriana de maior impacto para a humanidade (WHO, 2020a). O Relatório Global de Tuberculose da Organização Mundial de Saúde estima que em 2019, 10 milhões de pessoas (variação, 8,9-11,0 milhões) desenvolveram TB e destas, aproximadamente 1,2 milhão morreram e 208.000 mortes foram atribuídas à coinfecção TB-Vírus da Imunodeficiência Humana (HIV) (WHO, 2020b). No entanto, a doença é amplamente subnotificada e, essas amplas variações são indicativas dos desafios de diagnóstico e deficiência nos sistemas de vigilância em saúde pública (KOCH et al., 2021). A TB é um desafio global e grande parte dos casos estão concentrados em áreas onde a pobreza e a alta densidade populacional se sobrepõem (WHO, 2020a).

Cerca de um quarto da população mundial está infectada com *Mycobacterium tuberculosis* (WHO, 2020a). Todos os humanos são potencialmente susceptíveis à TB e cerca de 90% das pessoas que desenvolvem a doença são adultos. Há mais casos entre homens do que mulheres; e daqueles que adoeceram com TB em 2019, 87% estavam em 30 países com alta carga de TB. No Brasil, a incidência de TB tem aumentado lentamente nos últimos anos (WHO, 2020b).

O Brasil está na lista composta por 30 países que têm alta taxa de incidência da doença e junto a países como Rússia, Índia, China e África do Sul, compõe o BRICS, comportam 49% da carga mundial de TB e mais de 60% da carga de tuberculose multirresistente (WHO, 2019). A TB é um agravo transmissível de grande relevância no Brasil e, particularmente, no estado do Rio de Janeiro, por ser o segundo estado a nível nacional em casos confirmados, notificados no Sistema de Informação de Agravos de Notificação (SINAN), segundo dados do Ministério da Saúde/SVS - Sistema de Informação de Agravos de Notificação - Sinan Net (BRASIL, 2020).

## 2.3 Biomodelos Experimentais da Tuberculose

### 2.3.1 Primatas não humanos

Devido à alta similaridade genética com humanos, os PNHs modelam com precisão todos os aspectos da TB e são considerados como biomodelo padrão-ouro nas infecções experimentais por *Mtb* (FOREMAN et al., 2017). Os PVM foram incorporados aos estudos da TB pela primeira vez no final dos anos 1950, quando surgiu a hipótese de que a susceptibilidade à infecção natural poderia ser utilizada para estudar a terapêutica e a imunidade natural e induzida pela vacina à infecção (SCHMIDT, 1956; CLARKE, 1968; GOOD, 1968; SCHMIDT, 1966).

Os macacos rhesus (*M. mulatta*) foram inicialmente a espécie dominante como modelo da TB, em grande parte devido à sua relativa facilidade de manutenção e reprodução em cativeiro (BARCLAY et al. 1970; BARCLAY et al., 1973; GOOD, 1968; JANICKI et al. 1973). Os primeiros estudos utilizando macacos cynomolgus (*M. fascicularis*) estabeleceram um modelo de TB crônica ao usar doses muito baixas (10 unidades formadoras de colônias) (WALSH et al. 1996). Posteriormente, as fases latente, ativa e de reativação, ou seja, infecção latente progredindo para doença ativa, foram descritas nesta espécie como sinônimos de TB humana (CAPUANO et al. 2003; LIN et al. 2009). O modelo cynomolgus desde então foi rapidamente explorado para estudos de vacinas (OKADA et al. 2007; REED et al. 2009; MEHRA et al. 2013). À medida que os estudos delineiam as diferenças sutis na progressão da infecção por *Mycobacterium tuberculosis* (*Mtb*) nessas duas espécies, está se tornando cada vez mais claro que ambas fornecem informações sobre os diferentes aspectos da doença humana (FOREMAN et al., 2017).

Estudos experimentais demonstraram que saguis (*Callithrix* spp.) são altamente suscetíveis à inoculação por via intratraqueal com *Mtb* (VIA et al., 2013; CADENA et al., 2016). VIA et al., (2013) avaliaram a suscetibilidade dos saguis às micobactérias *Mtb* e *M. africanum* de diferentes virulências: o isolado clínico virulento K04 e a cepa Erdman, menos virulenta de *Mtb* (CDC1551) e *M. africanum*. De acordo com os autores, os saguis desenvolveram tuberculose (TB) que progrediu para doença sistêmica e extensa, recapitulando algumas das manifestações clínicopatológicas da TB humana. De acordo com SCANGA; FLYNN (2014) os saguis apresentam determinadas vantagens na modelagem da TB em relação aos primatas do Velho Mundo (PVM), como redução do peso, tamanho corporal e custos da experimentação, além da grande facilidade do manejo.

### 2.3.2 Camundongos

Os camundongos são relativamente resistentes ao *Mtb* e normalmente não desenvolvem TB similar aos humanos (SAKAMOTO, 2012). Apesar dos camundongos serem infectados por aerossol, as características anatômicas do trato respiratório superior afetam a dose final administrada aos pulmões (REZNIK, 1990). Além disso, os camundongos podem abrigar alta concentração de *Mtb* nos pulmões sem mostrar sinais clínicos (MCMURRAY, 2001), não tossem, não formam lesões cavitárias, tornando-os um modelo pobre para estudos com TB (KARLSSON, 1988).

### 2.3.3 Cobaias

As cobaias (*Cavia porcellus*) desenvolvem respostas a infecção por *Mtb* e reproduzem muitos dos aspectos da infecção humana, como granulomas caseosos e mineralizados, lesões pulmonares primárias e hematogênicas, formação de cápsula fibrosa e

disseminação hematógena (MCMURRAY, 1994). A cavitação pode ocorrer com infecções crônicas de baixas doses de cobaias (YAMAMURA, 1958). As cobaias são altamente vulneráveis à infecção, sendo necessária apenas uma baixa dose de micobactérias inaladas, tornando-as adequadas para estudos de transmissão aerógena de *Mtb* e como animais sentinela (RILEY, 1957). As cobaias, no entanto, não são adequadas para estudos de latência porque são hospedeiros muito suscetíveis e frequentemente desenvolvem doença progressiva crônica com extensa destruição de tecido após infecção por uma dose muito baixa (BEHAR, 1999).

### 2.3.4 Coelhos

Coelhos (*Oryctolagus cuniculus*) infectados com *Mtb* desenvolvem granulomas caseosos e formam lesões cavitárias (MANABE et al. 2003). Coelhos são relativamente resistentes à infecção por *Mtb*, no entanto, este modelo é útil no estudo de estados latentes da TB. Os coelhos precisam ser imunossuprimidos experimentalmente, pois não reativam a doença espontaneamente (MANABE et al., 2008).

## 2.4 Estágios da Doença

A TB é a principal causa infecciosa de mortalidade em humanos em todo o mundo, em parte devido a uma compreensão limitada de seu amplo espectro patogênico de infecção e manifestação da doença (DRAIN et al., 2018). Historicamente, os testes diagnósticos e o tratamento com medicamentos têm se concentrado em abordar dois estados da doença: infecção latente de tuberculose (ILTB) ou tuberculose ativa (TBA) (DRAIN et al., 2018). Organismos infectados com tuberculose (TB) podem progredir rapidamente para a doença ativa, entretanto, a grande maioria desenvolve uma infecção latente e permanece em risco de progressão para a doença ativa, ou seja, para o estágio de reativação (CADENA et al., 2017).

Para indivíduos com infecção latente de TB, o hospedeiro mantém uma relação dinâmica com *Mycobacterium tuberculosis* (*Mtb*) por meio da regulação do sistema imunológico inato e adquirido (BARRY et al., 2009; SCHWANDER et al., 2011). Há significativas diferenças entre primatas não humanos (PNHs) com tuberculose ativa (TBA) e com infecção latente de tuberculose (ILTB), incluindo o grau de patologia e carga bacteriana (CADENA et al., 2017). Alguns PNHs com doença clinicamente ativa apresentam pneumonia granulomatosa com cavitação, e doença extrapulmonar, enquanto outros PNHs podem apresentar manifestações limitadas aos linfonodos torácicos e um único lobo pulmonar (LIN et al., 2009).

De acordo com DRAIN et al., (2018), apesar da infecção latente de tuberculose ser uma infecção com *Mtb* viável, não é esperado que ocorra progressão para tuberculose ativa em um futuro próximo, na ausência de qualquer comprometimento imunológico significativo. Isso representa um análogo mais conceitual à definição atual da OMS, que considera a infecção latente de tuberculose como tendo evidência de infecção por micobactérias do Complexo *Mycobacterium tuberculosis* e nenhuma evidência clínica, radiológica ou microbiológica de tuberculose ativa (WHO, 2017; WHO 2018). Atualmente, não há uma maneira direta de confirmar infecção latente de tuberculose ou sua carga microbiológica, pois os testes existentes inferem ILTB com base em uma resposta de células T ou antígenos semelhantes à tuberculose (DHEDA et al., 2016).

Uma vez estabelecida a infecção, as vias pelas quais a doença pode progredir naturalmente incluem a latência que é o curso mais comum, progressão rápida ou lenta através de doença subclínica para tuberculose ativa, ou ainda um período de ciclos heterogêneos que podem preceder o desenvolvimento de doença sintomática ou eventual

resolução da doença (DRAIN et al., 2018). A tuberculose ativa e com infecção latente de tuberculose (ILTB), incluindo o grau da lesão é caracterizadas por *Mtb* viável que causa sintomas clínicos com anormalidades consistentes com a fase ativa da doença. Isso permaneceria consistente com a definição atual da OMS, que considera a TB ativa como "pacientes sintomáticos com evidências de *Mtb* na lesão" (WHO, 2010).

Após uma infecção primária, ocorre um retardo na resposta imune e, portanto, o volume bacilar que atinge os linfonodos de drenagem é maior do que na presença de uma resposta imune anterior (CARDONA, 2016). Os termos reativação e tuberculose pós-primária são usados indistintamente para conotar que a tuberculose primária é seguida por um período variável de latência clínica, após o qual a tuberculose se desenvolve, e contribui para a patogênese e manifestações clínicas. A reativação da tuberculose pode ocorrer após um evento imunossupressor, desenvolvimento de condições comórbidas ou mesmo ocorrer sem um antecedente específico. O pulmão é o principal órgão afetado em casos de reativação da tuberculose (ELLNER, 2012).

## 2.5 Sinais Clínicos

Os sinais clínicos de tuberculose em primatas não humanos variam de acordo com a localização e a gravidade da infecção (SIMMONS; GIBSON 2012). Quando presentes, os sinais clínicos podem ser inespecíficos, porém, frequentemente os primatas são encontrados mortos sem história clínica prévia (RENQUIST; WHITNEY, 1978). Conforme a doença progride, os PNHs demonstram dispneia aos esforços, letargia, anorexia e perda crônica de peso (SIMMONS; GIBSON 2012). Hepatomegalia palpável e esplenomegalia são relatadas em alguns casos.

Em um surto de *Mbo* em uma colônia de macacos rhesus e cynomolgus, observou-se que os macacos rhesus eram mais propensos a exibir sinais clínicos, incluindo tosse persistente e inapetência, do que macacos cynomolgus (GARCIA et al., 2004). De acordo com VIA et al. (2013), a perda progressiva de peso foi o sinal clínico mais comum em saguis infectados experimentalmente e que desenvolveram tuberculose. Além disso, os autores observaram pirexia de baixo grau, desidratação, anorexia e taquipneia.

## 2.6 Patogênese

A patogênese da tuberculose é altamente complexa e dinâmica (LENAERTS; BARRY; DARTOIS, 2015). A cepa infectante, bem como a via de infecção dos microorganismos pertencentes ao Complexo *Mycobacterium tuberculosis* podem impactar significativamente o curso e a manifestação da doença (PENA et al., 2015). A via mais comum da infecção é a aerógena, por meio de inalação de micropartículas contendo bacilos infectantes. A dose infecciosa para primatas não humanos é bastante variável e poucos bacilos já são considerados biologicamente relevantes para o curso da doença (DANNENBERG, 1978).

LIN et al. (2014) ao conduzirem um estudo com saguis infectados com *Mtb*, demonstraram que as lesões podem variar substancialmente dentro de um hospedeiro. Além disso, este estudo sugere que a heterogeneidade da lesão é mediada pela imunidade adaptativa do hospedeiro. As interações patógeno-hospedeiro dentro das lesões formam um processo dinâmico, provavelmente influenciado por mediadores pró-inflamatórios que induzem diferentes respostas teciduais nos organismos (LENAERTS; BARRY; DARTOIS, 2015).

Ao serem inalados e atingirem o espaço aéreo, os bacilos são fagocitados por macrófagos alveolares (SIMMONS; GIBSON 2012). Esses macrófagos são estimulados a produzir citocinas e quimiocinas pró-inflamatórias, levando ao recrutamento de mais

leucócitos para o local da infecção. Os neutrófilos e monócitos chegam primeiro, fagocitam bactérias adicionais, secretam mais citocinas e quimiocinas e começam a organizar o granuloma inicial (SAKAMOTO, 2012). O *Mycobacterium tuberculosis* (*Mtb*) recém fagocitado reside em um fagossomo e então as micobactérias bloqueiam a maturação, a fusão lisossomal e a acidificação da vesícula (ARMSTRONG; HART, 1975). O fagossomo micobacteriano retém as características dos endossomos iniciais, enquanto exclui os marcadores endossômicos tardios (CLEMENS; HORWITZ, 1996).

Se esses macrófagos não tiverem sucesso em destruir os bacilos, um foco central de necrose caseosa se desenvolverá no centro da área afetada (ELLNER, 2012). Neste local, será formado um granuloma bem organizado, consistindo de um foco de necrose caseosa circundado por histiócitos íntegros e degenerados, macrófagos epitelioides, macrófagos espumosos, linfócitos, variável número de células gigantes multinucleadas do tipo Langhans e, por vezes, pode ser delimitado por cápsula fibrocolagenosa (SAKAMOTO, 2012). Lesões primárias que contêm o patógeno frequentemente se desenvolvem como lesões granulomatosas mineralizadas, que são consideradas relativamente estáveis e menos dinâmicas do que outros tipos de lesão (ULRICHS; KAUFMANN, 2011).

As lesões necróticas representam dano irreversível no tecido afetado. Posteriormente, ocorre mineralização, fibrose e, às vezes metaplasia óssea (BASARABA, 2008). A mineralização distrófica é um processo patológico associado à deposição intra e extracelular de sais de cálcio, fósforo e magnésio em locais com necrose caseosa (WU-WONG et al., 2006). A mineralização é um processo progressivo e é considerada junto com a fibrose como uma resposta tecidual clinicamente favorável (BASARABA, 2008), enquanto a mineralização incompleta é desfavorável, uma vez que a necrose da lesão residual pode abrigar bacilos viáveis tolerantes a drogas (RIDLEY; RIDLEY, 1987).

O granuloma é mantido por uma resposta de hipersensibilidade tardia (tipo IV), mediada por linfócitos produtores de Interferon Gama (IFN-γ), à presença persistente de antígenos e lipídeos imunoestimuladores, pois *Mycobacterium tuberculosis* (*Mtb*) pode sobreviver dentro dos macrófagos e extracelularmente dentro do granuloma (MCCUNE et al., 1966). O granuloma é considerado uma área hipóxica em primatas não humanos (VIA et al., 2008), isto é uma consideração importante a favor do metabolismo micobacteriano e que reduz a eficácia da terapia antibiótica (MCMURRAY, 2001).

A reação granulomatosa formada no local primário de implantação do bacilo é denominada foco de Ghon. Os bacilos podem se espalhar antes da formação desses granulomas organizados através da drenagem linfática para os linfonodos mediastínicos, resultando em linfangite granulomatosa e linfadenite, que, junto com o Complexo de Ghon, é chamado de complexo primário de Ranke (SAKAMOTO, 2012). A disseminação hematógena no pulmão ou para outros órgãos também pode ocorrer. Curiosamente, os lobos pulmonares superiores do humano favorecem o crescimento bacilar devido à maior pressão de oxigênio e respostas imunológicas retardadas (PARK et al., 1992).

Com o passar do tempo, a necrose associada ao alto teor de lipídeos e proteínas dos macrófagos mortos resultam na aparência caseosa macro e microscópica (SAKAMOTO, 2012). Os granulomas caseosos são compostos predominantemente por necrose e debris celulares, lipídeos derivados do hospedeiro, e uma análise da regulação gênica nas células mostra desregulação do metabolismo lipídico do hospedeiro causada por *Mtb* (KIM et al., 2010). Na maioria dos casos de TB observam-se lesões pulmonares com morfologia heterogênea (KAPLAN et al., 2003), ou seja, cada granuloma contém bacilos em diferentes estágios de latência e reativação, o que caracteriza alterações teciduais distintas (FENHALLS et al., 2000).

Em biomodelos que desenvolvem lesões caseosas necróticas, como os primatas não humanos, bem como na tuberculose humana, o número de bacilos por lesão é considerado

relativamente reduzido, exceto para lesões liquefativas e cavitárias. Em primatas do Velho Mundo (PVM) com tuberculose ativa, observa-se de 0 a 75% de lesões estéreis (bacilos ausentes no tecido), enquanto 20 a 100% das lesões eram estéreis em macacos infectados de forma latente, mostrando que a esterilização da lesão não é apenas uma característica da doença latente (LENAERTS et al., 2015).

Cavitação é o processo pelo qual o granuloma caseoso é liquefeito e liberado através de uma via aérea erodida ou pelo espaço pleural (LENAERTS; BARRY; DARTOIS, 2015). Esse processo se dá por hidrólise do granuloma caseoso sólido. Embora o mecanismo não seja totalmente conhecido, foi demonstrado que as enzimas hidrolíticas (WEISS et al., 1954; ROJAS-ESPINOSA et al., 1974), a falta de vascularização e a resposta imomediada tardia desempenham papel importante neste processo. A presença e extensão da doença cavitária são frequentemente correlacionados com um prognóstico desfavorável e desenvolvimento de resistência bacteriana (ABER; NUNN, 1978).

Curiosamente, os tecidos extrapulmonares podem estar persistentemente infectados com microorganismos do Complexo *Mycobacterium tuberculosis* (HERNANDEZ-PANDO et al., 2000; NEYROLLES et al., 2006). Em humanos, quase 15% dos casos de reativação da tuberculose ocorrem em locais extrapulmonares, como sistema nervoso central, pele e trato geniturinário, sem lesões pulmonares associadas (BACKER et al., 2006). O primeiro local de disseminação extrapulmonar é geralmente o linfonodo mediastínico por meio de vasos linfáticos aferentes de drenagem. A disseminação para outros órgãos extrapulmonares é hematógena ou através do trato gastrointestinal a partir de bacilos deglutidos (LIN et al., 2006). As diferenças podem ser específicas do tecido, relacionadas ao tipo e distribuição da vascularização tecidual (BASARABA, 2008). Na ausência de progressão, um indivíduo infectado pode permanecer assintomático com bacilos existindo em um estado latente, possivelmente controlado pelo sistema imunológico do hospedeiro (SAKAMOTO, 2012).

## 2.7 Patologia

### 2.7.1 Macroscopia

Frequentemente, em primatas não humanos (PNHs) com tuberculose (TB) observa-se o tubérculo típico, um granuloma com estrutura característica. Os granulomas apresentam-se como nódulos firmes, branco-amarelados ou acinzentados, de diferentes tamanhos. Lesões focais ou multifocais a coalescentes são observadas no pulmão, que é o órgão mais comumente afetado. Os tubérculos podem se estender até a pleura torácica ou traqueia. Os linfonodos traqueobrônquicos podem estar muito aumentados com áreas caseosas na superfície de corte (MÄTZ-RENSING; LOWENSTINE, 2018).

Os estágios avançados da doença são caracterizados pela disseminação para locais extrapulmonares. Os granulomas são frequentemente vistos no baço, rim, fígado e em diferentes linfonodos. A disseminação para outros órgãos varia entre os PNHs e não se correlaciona necessariamente com a gravidade do envolvimento pulmonar (SIMMONS; GIBSON 2012).

### 2.7.2 Microscopia

Estudos em PNHs demonstraram que os granulomas tuberculosos não são estruturas uniformes e, em vez disso, apresentam uma grande variedade de apresentações histopatológicas (SCANGA; FLYNN, 2014). Vários tipos de lesões podem coexistir até mesmo no mesmo indivíduo. Essas mesmas características podem ser vistas em PNHs infectados experimentalmente (LIN et al., 2009; FLYNN; KLEIN, 2011). Segundo MÄTZ-

RENSING; LOWENSTINE (2018), histologicamente os granulomas típicos podem ter tamanhos variados, com um centro com debris picnóticos ou material proteico e amorfo, que em PNHs raramente é mineralizado. Esses centros necróticos são circundados por macrófagos epiteloides, linfócitos, neutrófilos íntegros e degenerados e células gigantes multinucleadas, geralmente do tipo Langhans.

De acordo com SIMMONS; GIBSON (2012), diferentemente de outras espécies animais, uma cápsula fibrosa geralmente não é encontrada em PNHs. O número de bacilos álcool-ácido resistentes dentro de lesões granulomatosas pode variar consideravelmente, mas frequentemente existem apenas alguns organismos evidenciados pela histoquímica de Ziehl-Neelsen. Lesões cavitárias em biomodelos animais geralmente se desenvolvem como uma continuação da infecção primária. Geralmente as cavidades progridem da necrose caseosa para a necrose de liquefação, substituindo o tecido pulmonar parenquimatoso (BASARABA, 2008).

Em lesões crônicas, o *Mycobacterium tuberculosis* (*Mtb*) se localiza predominantemente em macrófagos espumosos na periferia das lesões (KAPLAN et al., 2003). Esses macrófagos espumosos predominam nos estágios avançados da TB e são induzidos por micobactérias virulentas em resposta aos ácidos micólicos presentes na parede celular (PEYRON et al., 2008). Essas células têm atividade microbicida e fagocítica reduzidas e, portanto, acredita-se que forneçam um reservatório rico em nutrientes para a persistência de *Mtb* (SAKAMOTO, 2012).

## 2.8 Diagnóstico

### 2.8.1 Bacteriológico

A família Mycobacteriaceae possui um amplo espectro de bactérias que apresentam diferentes dinâmicas de crescimento em cultura e distintas características de patogenicidade (FONG, 2020). O cultivo e isolamento bacteriano são considerados o padrão ouro do dagnóstico de TB em PNHS (DUNN; STARKE; REVELL, 2016), entretanto, pode ser difícil especialmente no início da doença, quando um número baixo de micobactérias está presente (SIMMONS; GIBSON, 2012). Esse processo pode ser prolongado devido à natureza de crescimento lento dos organismos do Complexo *Mycobacterium tuberculosis* (CMTB) e pode levar semanas a vários meses para ser concluído (MANSFIELD; FOX, 2019).

Para o diagnóstico *ante-mortem*, *swabs* orais, lavados gástricos e amostras de fezes podem ser utilizados como amostras para cultivo micobacteriano em primatas não humanos (MANSFIELD; FOX, 2019). Essas amostras podem ser semeadas em meio Lowenstein–Jensen (L–J), que é o mais tradicionalmente usado para isolar cepas humanas de *Mycobacterium tuberculosis* (*Mtb*) e a maioria das outras micobactérias (ACHARYA et al., 2020). Tanto *Mtb* quanto *M. bovis* (*Mbo*) crescem em meio artificial a 37° C, mas *Mtb* prefere meio com glicerol, enquanto o glicerol inibe o crescimento de *Mbo* (SIMMONS; GIBSON 2012). A identificação de *Mtb* em culturas positivas pode ser realizada por testes bioquímicos para Niacina, Arilsulfatase, Vermelho neutro, Catalase-peroxidase, Amidase e nitrato redutase após a incubação de 2 a 3 semanas (FORBES; SAHM; WEISSFELD, 2000).

### 2.8.2 Molecular

A reação em cadeia pela polimerase (PCR) é o método de diagnóstico molecular mais importante usado para diagnosticar tuberculose (NESHANI et al., 2018). Nos últimos anos, várias sequências alvo foram usadas para diagnosticar os microrganismos do Complexo

*Mycobacterium tuberculosis* (CMTB) por PCR, como IS6110, mpb64, devR, hsp65, 38 KDa (pstS1), 30 KDa (fbpB), esat6, cfp10, gene 16S rDNA (rrs) e rpoB (HALDAR et al., 2011; RAJ et al., 2016). Os testes de diagnóstico baseados nesses alvos têm alta sensibilidade e especificidade para a discriminação dos microrganismos pertencentes ao CMTB (NESHANI et al., 2018).

Testes moleculares para o diagnóstico de tuberculose em primatas não humanos, como a PCR têm como vantagem a rápida velocidade de execução, podendo estar disponíveis em questão de horas, em vez de semanas necessárias para o cultivo (MANSFIELD; FOX, 2019). Além disso, a amplificação de *primers* específicos através da PCR para microorganismos do CMTB em tecidos coletados na necropsia pode levar a um diagnóstico mais precoce e preciso do que a cultura (BRAMMER et al., 1995). O ensaio Xpert® MTB / RIF é baseado na amplificação usando um PCR multiplex em tempo real da região central de 81 pares de bases do gene rpoB específico para *Mycobacterium tuberculosis* e mutações determinantes de resistência à rifampicina (LAWN; NICOL, 2011). Em situações com baixa carga de micobactérias, o GeneXpert® MTB / RIF é associado à sensibilidade limitada na detecção de *Mtb* (SOHN et al., 2014), e com elevada quantidade de resultados falsos positivos (AREND; SOOLINGEN, 2018) e eficiência reduzida em comparação com a PCR convencional (SOHN et al., 2014).

### 2.8.3 Imunológico

#### 2.8.3.1 Teste tuberculínico

O diagnóstico *ante mortem* da tuberculose é tradicionalmente baseado no teste tuberculínico intradérmico (SIMMONS; GIBSON, 2012). O princípio deste teste é baseado na clássica resposta de hipersensibilidade tardia (tipo IV) à proteína purificada derivada (PPD) tuberculínica, que é uma mistura de antígenos derivados do *Mycobacterium tuberculosis* (LIN et al., 2008). O teste tuberculínico consiste na administração de 0,10 ml de (tuberculina) por via intradérmica na pálpebra ou na pele abdominal. O local da inoculação é observado às 24, 48 e 72 horas pós-inoculação e a reação tecidual, se houver, é mensurada. Observa-se uma reação granulomatosa no local da aplicação e eritema, edema e ptose, que são indicativos de uma reação suspeita ou positiva (MCLAUGHLIN; MARRS, 1978).

O teste tuberculínico é limitado em sua eficácia, pois animais com doença em estágio inicial ou avançado podem apresentar reações falso negativos (SIMMONS; GIBSON, 2012). Doenças concomitantes em primatas não humanos, como o sarampo, ou mesmo uma história recente de vacinação contra o sarampo, também podem resultar em uma reação falso-negativa devido à supressão da resposta imune mediada por células (MCLAUGHLIN; MARRS, 1978). Falsos positivos podem resultar da exposição ao adjuvante completo de Freund ou de trauma devido à administração inadequada do teste ou reatividade não específica ao veículo (PIERCE; DUKELOW, 1988).

#### 2.8.3.2 Interferon gama

Devido aos resultados falsos positivos e falsos negativos do teste tuberculínico, outros métodos foram desenvolvidos para diagnosticar a infecção clínica em humanos e em primatas não humanos (LIN et al., 2008). O Interferon Gama (IFN-γ é uma citocina de células T que ativa macrófagos para produzir espécies reativas de oxigênio e nitrogênio, é provavelmente a citocina mais importante na resposta imune à TB. Esta citocina é secretada principalmente por linfócitos citotóxicos Th1 e CD8þ, linfócitos T *natural killer*, células T,

células apresentadoras de antígenos e, em menor extensão, células B (MARTÍNEZ et al., 2009).

Existem dois métodos principais pelos quais o IFN-γ pode ser mensurado (LIN et al., 2008). De acordo com GARCIA et al. (2004) no primeiro método a quantidade de IFN-γ é mensurada por ensaio de imunoabsorção enzimática (ELISA). O segundo método quantifica o número de células produtoras de IFN-γ, em vez de uma quantidade total de IFN-γ produzida no método baseado em ELISA (LIN et al., 2008).

### 2.8.4 Imuno-histoquímica

A presença de antígenos de *Mycobacterium tuberculosis* (*Mtb*) no tecido pode ser avaliada por meio da técnica de imuno-histoquímica (IHQ) (KARIMI et al., 2014). A IHQ usando anticorpos específicos tem o potencial de revelar a presença de bacilos nos tecidos analisados, mesmos que estes não possuam a parede celular bacilar intacta (MUSTAFA et al., 2006). Desse modo, a IHQ é considerada mais sensível do que a coloração de Ziehl-Neelsen para microorganismos ácido-resistente e tem sido empregada no diagnóstico da TB em múltiplas espécies (MUSTAFA et al., 2006; KARIMI et al., 2014; RIBEIRO et al., 2017; EHLERS et al., 2020).

## 2.9 Tratamento

O tratamento de primatas não humanos (PNHs) com tuberculose (TB) em alguns casos é eficaz (SIMMONS; GIBSON, 2012). A terapia unicamente com isoniazida como preventivo ou como agente terapêutico é considerada ineficaz (BURKHOLDER et al., 1967; CLARKE; SCHMIDT, 1969). A terapia multimodal usando duas ou mais moléculas tem sido bem empregada em PNHs com TB (MCCLURE, 1980; WARD et al., 1985). O uso de múltiplos agentes diminui a possibilidade de desenvolver resistência a antibióticos e permite agrupar agentes que atacam a micobactéria em diferentes estágios de seu ciclo de vida (WOLF et al., 1988).

Combinações eficazes de drogas incluem isoniazida e estreptomicina (FREMMING et al., 1957; INDZHILA et al., 1977; WARD et al., 1985), isoniazida e ácido aminossalicíclico (MCCLURE, 1980), e isoniazida, etambutol e rifampicina (WOLF et al., 1988). A falha em identificar o agente etiológico, bem como em determinar a sensibilidade aos antibióticos pode levar ao fracasso do tratamento (HABERLE, 1970; DILLEHAY; HUERKAMP, 1990).

## 2.10 Prevenção e Controle

Programas de saúde e segurança ocupacional e programas de saúde preventiva das colônias são essenciais para a prevenção e controle da tuberculose em PNHs (CADENA et al., 2016). Procedimentos eficientes de quarentena, práticas de manejo e procedimentos de monitoramento e proteção de pessoal e primatas não humanos são úteis para prevenir surtos de TB em uma colônia (BUSHMITZ et al., 2008). Exames completos e específicos para o diagnóstico de tuberculose (TB) devem ser realizados constantemente na colônia (MAZUREK; VILLARINO, 2003; PAI et al., 2004).

De acordo com BUSHMITZ et al., (2008), quando um diagnóstico positivo de TB é feito em um PNH, ele deve ser imediatamente eutanasiado e a carcaça deve ser encaminhada à necropsia. Os recintos devem ser higienizados e os PNHs restantes devem ser colocados sob um período inteiro de quarentena ou serem eutanasiados.

## 3. CAPÍTULO 1

# Pulmonary granuloma is not always the tuberculosis hallmark: pathological findings of different tuberculosis stages in New and Old World Nonhuman Primates naturally infected with the *Mycobacterium tuberculosis* Complex

Asheley H. B. Pereira[1], Claudia A. A. Lopes[2], Thalita A. Pissinatti[2], Ana C. A. Pinto[2], Daniel R. A. Oliveira[3], Gabriel M. Leal[2], Luiz C. M. Oliveira[3], Paulo Redner[3], Bruna E. P. Barbosa[4], Silvia B. Moreira[5], Alcides Pissinatti[5,6], Fernanda H. Maruyama[7], Luciano Nakazato[7], Valéria Dutra[7], Daniel G. Ubiali[1]

[1] Pathological Anatomy Sector, Department of Epidemiology and Public Health, Veterinary Institute, Federal University Rural of Rio de Janeiro (UFRuralRJ), Highway BR-465 Km 7, Ecologia, Seropédica, RJ 23890-000, Brazil. Corresponding Author: Daniel G. Ubiali, Tel: 5521998095217. Email: danielubiali@ufrrj.br

[2] Nonhuman Primate Breeding Service (Scprim)/Institute of Science and Technology in Biomodels (ICTB), Oswaldo Cruz Foundation (Fiocruz), Av. Brasil, 4365 - Manguinhos, Rio de Janeiro - RJ, 21040-360, Brazil

[3] National Laboratory for Tuberculosis and Other Mycobacterioses, Professor Hélio Fraga Reference Center, National School of Public Health, Oswaldo Cruz Foundation.

[4] Residency Training Program in Wildlife Medicine and Conservation, Veterinary Institute, UFRuralRJ

[5] Rio de Janeiro Primatology Center (CPRJ/INEA), Guapimirim/RJ, 25940-000, Brazil

[6] Serra dos Órgãos University Center, Teresópolis, RJ, Brazil

[7] Microbiology and Molecular Biology Laboratory, Federal University of Mato Grosso, Cuiabá, Mato Grosso, Brazil

## Abstract

Herein we present the pathological findings of different tuberculosis stages in Old and New World monkeys kept under human care in Rio de Janeiro, Brazil and naturally infected with *Mycobacterium tuberculosis* Complex. Fifteen nonhuman primates from five different colonies were incorporated into the study. There are 60% (9/15) Old World Monkeys and 40% (6/15) New World Monkeys. According to the gross and histopathologic findings, the lesions in nonhuman primates of this study are classified into the chronic-active, extrapulmonary, early-activation or latent-reactivation tuberculosis stage. Among the Old World Monkey, 66.7% (6/9) of nonhuman primates, all rhesus monkeys (*Macaca mulatta*), showed severe granulomatous pneumonia. In all Old World Monkeys cases, typical granulomas were seen in at least one organ regardless of the stage of the disease. In the New World Monkeys, the typical pulmonary granulomas were seen in 16.7% (1/6) of the cases, just in the latent-reactivation stage in Uta Hick's Bearded Saki (*Chiropotes utahickae*). In this study, 66.7% (6/9) of Old World Monkeys (OWM) and 83.3% (5/6) of New World Monkeys (NWM) showed pulmonary changes at the histological evaluation. The tuberculosis diagnosis in the nonhuman primates in this study was based on pathological, immunohistochemical, molecular, and bacteriological culture. Although the typical presentation was observed in some cases, the absence of pulmonary granuloma did not exclude the tuberculosis occurrence in nonhuman primates of the Old and New World. Tuberculosis should be included as a cause of interstitial pneumonia with foamy macrophages infiltration in the New World nonhuman primates. Due to the high sensitivity of immunohistochemistry with Anti-*Mycobacterium tuberculosis*, we suggest the addition of this technique as a diagnostic tool of tuberculosis in the nonhuman primates even when the typical changes are not seen.

Keywords: Tuberculosis, Nonhuman Primates, pathological findings.

## Introduction

*Mycobacterium tuberculosis* complex (MTBC) refers to a group of mycobacteria encompassing nine members of closely related species that cause tuberculosis (TB) in

animals, including humans (Kanabalan et al., 2021). Tuberculosis is consistently the most impactful bacterial disease to affect humanity, with a quarter of all humans infected and responsible for the most significant number of infection-related deaths and long-term disability (WHO, 2020a). In 2019, an estimated 10 million people were having health issues with TB worldwide, and it was considered one of the top 10 causes of human death and the leading cause of a single infectious agent (WHO, 2020b). Tuberculosis is a chronic airborne disease that causes high morbidity and mortality in humans and nonhuman primates (NHPs), especially captive macaque monkeys (Warit et al., 2020).

Although particular species may be more or less susceptible to disease, all nonhuman primates can develop tuberculosis (Mätz-Rensing and Lowenstine, 2018). Due to the high genetic similarity with humans, NHPs accurately model all TB aspects and are considered the gold standard biomodel in experimental *M. tuberculosis* infection (Foreman et al, 2017). Infections in captive nonhuman primates by MTBC mainly occur due to direct contact with TB-infected humans through the inhalation of aerosolized bacteria (Ghodbane and Drancourt, 2013; Mätz-Rensing et al, 2015; Warit et al., 2020). Captive nonhuman primates develop an infection clinically indistinguishable from human tuberculosis (Kaushal et al., 2012; Kanabalan et al., 2021), and they recapitulate the full spectrum of infection outcome and pathology seen in humans (Scanga and Flynn, 2014).

The hallmark of tuberculosis is a typical granuloma presentation in any organ (Mätz-Rensing and Lowenstine 2018). According to Lin et al. (2009), in macaques with active-chronic TB, classical caseous granulomas, suppurative granulomas, nonnecrotizing granulomas, tuberculous pneumonia, and cavitary lesions were observed. The authors report latently infected monkeys present classic caseous lesions with mineralized granulomas and completely fibrotic pulmonary areas. In cases of rapidly progressive TB, the authors report a pattern of lesions consistent with a primary pulmonary disease with disseminated miliary, sometimes confluent small pulmonary granulomas, and extrapulmonary spread into the hepatic, splenic, and mesenteric tissue.

TB is well known and widely reported disease in laboratory Old World Monkeys (OWM), and most experimental studies have been performed in this nonhuman primate group (Schmidt 1956; Clarke, 1968; Walsh et al. 1996; Okada et al. 2007; Reed et al. 2009; Mehra et al. 2013). The natural occurrence in New World Monkeys (NWM) species is considered uncommon (Mätz-Rensing and Lowenstine 2018). Reports of Brazilian TB occurrence in nonhuman primates are scarce. Brazil is among the 30 countries with the

highest incidence of human tuberculosis worldwide (WHO, 2019) and in 2020, Rio de Janeiro state was considered the second state with the most cases of human tuberculosis (BRASIL 2020). We describe the pathological findings of different stages of *Mycobacterium tuberculosis* Complex infection in New and Old World Nonhuman Primates kept under human care in Rio de Janeiro, Brazil.

## Materials and Methods

### Animals and study area

This study was conducted in nonhuman primates kept under human care in Rio de Janeiro, Brazil. Fifteen nonhuman primates with a suspected or prior diagnosis of tuberculosis, regardless of species, sex or age, from five captive colonies were be included in the study. The epidemiological data about sex and age in each case were acquired with the colonies' technical veterinary.

The colony is the Nonhuman Primate Breeding Service (SCPrim) of the Biomodel Science and Technology Institute (ICTB) of the Oswaldo Cruz Foundation (Fiocruz), Rio de Janeiro, Brazil (2°52'39.06" S, 43°14'46.20" W). Currently, SCPrim has 510 rhesus monkeys (*Macaca mulatta*), 56 cynomolgus monkeys (*Macaca fascicularis*) and 123 squirrel monkeys (*Saimiri* sp.), of which 118 are *S. sciureus* and five *S. ustus*. The nonhuman primates are bred and maintained according to the guidelines of the National Council for the Control of Animal Experimentation, CONCEA (BRASIL, 2015), the Guide for the Care and Use of Laboratory Animals (National Research Council, NRC, 2003; 2011) and the Federation of European Laboratory Animal Science Associations (FELASA) (BALANSARD et al., 2019). Animal handling procedures were approved by the Ethics Committee on the Use of Animals (CEUA, Fiocruz) under license number LW-5/16 and additive terms. The management of wild fauna in the farm is also authorized by the Brazilian Institute for the Environment and Renewable Natural Resources (IBAMA) under number 556664, INEA-RJ.

All colonies receive filtered and treated water *ad libitum* through automatic stainless steel drinkers. The diet consists of extruded commercial feed (Nuvilab® Old World Monkeys and Nuvilab® Neotropical Primates, both from Quimtia, Colombo, Paraná, Brazil) in the morning, supplemented with horticultural items in the afternoon. The vegetables are routinely sanitized by submersion in a chlorinated solution at 0.02% for 20 minutes for parasitological and bacteriological control. The enclosures, built-in grid and masonry, are furnished with perches, drums and swings. Other food, physical or sensory environmental enrichment items are offered alternately (grains, herbs, fruit ice cream, popcorn, tire swing,

swimming pool on hot days, among others). All enclosures have a covered refuge area for animals to protect themselves from weather and other animals during a fight. The enclosures included an open environment with natural light and trees.

The rhesus colony has nine modules divided into two enclosures (A and B) with 64m$^2$ each and two modules divided into four enclosures (A, B, C and D) with 34m² each totaling 26 enclosures. In these enclosures, groups of five NHP on average are housed. Some enclosures house all-male groups, and there is an enclosure for older NHP. The monkeys are divided into family groups consisting of male and female alphas and other animals of different ages, with an average of 20 individuals per enclosure. The smaller cages house the primates or groups of all males separated to carry out population control of the colony or reserve breeding stock.

The cynomolgus colony is bred in a building consisting of five in-line enclosures measuring 7.35 m$^2$ each, containing part of the covered area and part of the uncovered area. The social groups are formed by males, females of different ages and offspring, with an average of 11 individuals per enclosure. There is also a group composed only of males, with 15 NHP. The Saimiri colony forms a complex of six modules divided into two enclosures (A and B) with an external area of 17.10 m$^2$ and an internal area (refuge) with 2.85 m$^2$, a total of 19.95 m$^2$ each. Social groups are distributed with an average of 11 animals per enclosure.

The colony of the Triage Center of Wild Animals (CETAS), in Seropédica, Rio de Janeiro state, Brazil (22°43'34.1"S 43°42'22.4"W). CETAS is a federal environmental organization that works with wildlife animal rescue, especially animals from smuggling. The goal is to rehabilitate and reintroduce many species in their natural habitat or redirect them to a definitive place, such as a zoo or a legal breeder. The center works approximately with 6,000 animals a year and is under the jurisdiction of the IBAMA. Installation of CETAS at approach time housed about 10 *Sapajus* sp. and 14 *Callithrix* sp., but that number changes drastically once the center receives and destinates animals every once in a while, as well as there could be deaths for many different reasons. The primates in the center mainly eat fruits, vegetables, boiled eggs and raw cattle hearts previously frozen. These items are washed with common sink water, and there is no control of the quality of this water. Because the center has limited money resources, it is not uncommon for the primates to be fed a limited variety of items for long periods.

The *Sapajus* spp. are kept in an exclusive block for larger animals, a medium-size enclosure 3 x 3,44 x 4 m has four in-line smaller enclosures inside, five on each side. Inside

each enclosure, the monkeys are kept according to affinity and proportion of males to females (approximately 1:2). In this study, there was no breeding control program, and all animals were intact. Inside their enclosure, there are perches (varying in size), as well as strings and hanging tires for their entertainment. There are no trees and/or plants.

The *Callithrix* sp. species are kept in individual bird cages or into families in a cement enclosure in a block composed of a line of eight enclosures. They are surrounded by psittacines and passerines' enclosures (on the right and the left, respectively). Each enclosure has about 17 $m^2$. These NHP have no tagging or any other type of marking, so it is almost impossible to track their origin once they arrive in the center after a while. In cases in which there are males and females together, the males are castrated. CETAS is short in staff, and the same keeper that handles the other animals (birds, reptiles and mammals) also handles the *Callithrix* and *Sapajus* species.

The colony of the Rio de Janeiro Primatological Center (CPRJ) of the Environmental State Institute (INEA) of Rio de Janeiro, Brazil (22º29'14.51" S, 42º 48'78" W), an institution devoted to the Conservation of endangered neotropical primates. The nonhuman primates are bred and maintained according to the CONCEA (BRASIL, 2015) and the Best Practice Guidelines for Callithrichidae, EAZA (AMSTERDAM, 2017). The breeding colony is authorized by the IBAMA, under number 4989806, INEA/RJ.

Currently, CPRJ has 350 neotropical primates, 276 of which are from the Callithrichidae family (genus *Callithrix, Cebuella, Mico, Saguinus* and *Leontopithecus*), 41 from Cebidae (genus *Sapajus, Saimiri* and *Cebus*), 3 Aotidae (genus *Aotus*), 15 Pitheciidae (*Callicebus, Plecturocebus, Pithecia, Cacajao* and *Chiropotes*) and 12 from Atelidae (genus *Alouatta* and *Ateles).* These primates are housed in family groups or pairs, with no public access, in 140 outdoor enclosures built in masonry and iron screen and furnished with perches and nest boxes or shelters. The enclosures are located in an open environment with access to natural sunlight and are protected from rain and cold wind. The CPRJ possesses an area of 270 hectares in the Parque Estadual dos Três Picos (PETP) in the Bioma Atlantic Forest.

The primates receive treated water *ad libitum* in stainless steel drinkers. The diet consists of extruded commercial feed (Nuvilab® Old World Monkeys and Nuvilab® Neotropical Primates, both from Quimtia, Colombo, Paraná, Brazil) in the morning, supplemented with horticultural items in the afternoon. Folivorous species also receive fresh leaves every day, and the insectivorous receive bugs and worms regularly. The vegetables

are routinely sanitized by submersion in a chlorinated solution at 0.02% for 20 minutes for parasitological and bacteriological control.

**Clinical Assessment**

At Nonhuman Primate Breeding Service (SCPrim/ICTB), the NHPs are physically restrained and sedated with an association of ketamine hydrochloride (Cetamin, Syntec®, Brazil) 10 mg/kg + midazolam hydrochloride (Teuto®, Brazil) 1mg/kg intramuscularly. After restraint, the primates are weighed, and clinical examination is performed, such as palpation of the abdomen and lymph nodes, examination of the oral cavity, skin, mucous membranes, pulmonary and cardiac auscultation, and temperature verification. Depending on the case, blood is collected for hemogram and biochemistry, sent to the ICTB laboratory.

In the Triage Center of Wild Animals (CETAS), in order to perform clinical and routine examinations, the *Callithrix* species are manually restrained (usually by towels or leather gloves), and *Sapajus* species are examined under sedation with ketamine + midazolam intramuscularly (the dose varying from 10mg/kg to 0,5mg/kg to 1mg/kg, respectively). These animals are also weighed, palpated and checked for abnormalities in heart/lungs auscultation, oral cavity, lymph nodes, skin, mucous membranes and temperature. Clinically ill patients can have blood withdrawn, feces collected, and x rays/ultrasound performed at the Veterinary Hospital at the UFRuralRJ.

At CPRJ, the NHP are captured with nets and physically restrained and sedated with ketamine hydrochloride (Cetamin, Syntec®, Brazil) 10 mg/kg + midazolam hydrochloride (Teuto®, Brazil) 1mg/kg intramuscularly. After restraint, the primates are weighed, and clinical examination is performed, such as palpation of the abdomen and lymph nodes, examination of the oral cavity and teeth, nostrils, ears, eyes, skin and fur, mucous membranes, genital, urinary and rectal openings, pulmonary and cardiac auscultation and temperature verification. Clinically ill animals can have biological samples analyzed or x-rays/ultrasounds performed.

**Necropsy and sample collection**

In the cases of death or euthanasia in nonhuman primates, the necropsy was realized by the technical responsible of the colony. The euthanasia of nonhuman primates from SCprim colonies was performed after sedation with ketamine hydrochloride 10mg/kg + midazolam hydrochloride 1m/kg and consist of intravenous injection of thiopental sodium 30-60 mg/kg with continuous infusion until the deep plane of anesthesia is reached, then progressing to coma and death. In the case of nonhuman primate euthanasia from CETAS

colony, the sedation was performed with ketamine 40mg/kg + xylazine 2mg/kg intramuscularly and, after the loss of consciousness and protective reflexes, subsequent intracardiac administration of potassium chloride 19,1%. These methods follow the CONCEA Normative Resolution 28/2015 and Brazilian Guide of Good Practices for Animal Euthanasia (CFMV 2013; BRASIL, 2015).

The necropsy of all nonhuman primates was performed by at least two veterinary pathologists of the Anatomy Pathology Sector of Federal University Rural of Rio de Janeiro (SAP/UFRuralRJ), Nonhuman Primate Breeding Service (SCPrim) or Primatology Center of Rio de Janeiro (CPRJ). During the procedure, personal protective equipment was used. A complete set of tissue samples as skin, skeletal muscle, tongue, thyroid, lymph nodes, trachea, lungs, heart, liver, spleen, kidneys, adrenals, stomach, intestines, pancreas, brain, and nerves were collected in each case and fixed in 10% buffered formalin solution. Fragments from multiple organs were collected in microtubes for referral to bacterial culture (N=6) and molecular biology (N=14).

**Histopathology and immunohistochemistry**

The fragments stored in formalin will be fixed for 24-48 hours and subsequently submitted to routine histological processing. Histological slides were stained with Hematoxylin and Eosin and then observed under an optical microscope. Histological sections with lesions morphologically compatible with tuberculosis were submitted to the Ziehl-Neelsen histochemical technique for acid-alcohol-resistant bacilli evidence. To determine the fibrous capsule of granuloma, sections of the lung were submitted to Trichrome Masson's histochemical technique.

At the immunohistochemistry technique, blocks were cut into 3 μm slices and mounted onto silane-coated microscope slides. Antigen retrieval was performed with 0.1% trypsin for 30 minutes in an oven at 37°C. The sections were then incubated in methanol: $H_2O_2$ solution (97%:3%) for 30 min to block nonspecific binding. The sections were set at 37°C for 2 hours with primary anti-*Mycobacterium tuberculosis* polyclonal antibodies (Bioscience Inc®, San Diego, CA, USA) at 1 in 300 dilutions or phosphate buffer saline (PBS) as the negative control. As a positive control, a case previously confirmed by PCR will be used, and as a negative control of the reaction, the primary antibody will be replaced by Buffered Saline Solution (PBS). Then, the sections will be submitted to EnVision™ secondary polymer (Dako®, Carpinteria, CA, USA) in an oven at 37ºC for 30 minutes. The development was done with 3,3-diaminobenzidine chromogen (DAB + Substrate

Chromogen System, DakoCytomation®, Carpinteria, California) for 2 minutes. Finally, the histological sections were counterstained with Harris hematoxylin and coverslipped. Were considered positive the cases in which structures morphologically compatible with *Mycobacterium* spp. bacilli were evidenced extracellularly or inside the cytoplasm of macrophages and multinucleated giant cells.

**Molecular Analysis**

The samples in RNA later were submitted to DNA extraction with glass beads, followed by phenol-chloroform, according to Sambrook and Russel (2001). In turn, the paraffin samples were extracted according to Shi et al (2004), adding glass beads. For the identification of CMTB, oligonucleotides INS1(5'CGTGAGGGCATCGAGGTGGC-3') and INS2(5'GCGTAGGCGTCGGTGACAAA-3') were used, which detect the genomic region IS6110 (Kolk et al., 1992). The amplified products were separated by electrophoresis in 1.5% agarose gel for 1 hour at 60 V, stained with Gel Red (Biotium) and visualized in ChemiDocTM XRS using ImageLabTM® software. For the identification of mycobacteria at the species level, oligonucleotides TB11 (5'-ACCAACGATGGTGTGTCCAT-3') and TB12 (5'-CTTGTCGAACCGCATACCCT-3') were used, which detect the one segment of the heat shock protein gene of 65 kDa (hsp65), according to Telenti et al. (1993). The amplified products were separated by electrophoresis in 1.5% agarose gel for 1 hour at 60 V, stained with Gel Red (Biotium) and visualized in ChemiDoc™ XRS using ImageLabTM® software. The PCR product was purified in the GFX™ PCR DNA and Gel Band Purification kit (GE Healthcare) and submitted to sequencing in the automatic ABI-PRISM 3500 Genetic Analyzer (Applied Biosystems).

For molecular diagnosis in live animals, oral swab, nasal swab, rectal and gastric lavage samples are collected. Swabs are collected without medium, and gastric and rectal lavage are performed with the aid of a probe and the use of 0.9% saline solution. Samples are kept refrigerated for up to two hours after collection and sent for laboratory analysis. After treatment and purification with NALC-NaOH (N-acetyl-L-cysteine-sodium hydroxide), the samples are analyzed using GeneXpert® (Cepheid, USA) commercial kits Xpert® MTB/RIF or Xpert® MTB/RIF Ultra, for MTBC complex detection and rifampicin resistance test.

**Bacterial culture**

Previously macerated tissues were cultured using the Ogawa-Kudoh method (Kudoh, Kudoh, 1974). All samples are also sent for direct smear microscopy with Ziehl-

Nielsen staining to identify acid-resistant bacilli and bacterial culture in Löwenstein-Jensen medium and incubated for up to 72 days at 37°C in a biological safety cabinet class 3. The *Mycobacterium tuberculosis* complex species in positive cultures were identified using an immunochromatographic assay with the MPT64 marker (Silva et al., 2017).

**Tuberculosis stage classification**

To determine the tuberculosis stages as active-chronic and latent-reactivation, the criteria established were followed by Capuano et al. (2003) and Lin et al. (2009). We suggest classifying the early-activation stage of tuberculosis according to clinical and morphological findings. There was no evidence of necrosis or granuloma formation in the lung parenchyma or any other organ analyzed to fulfill the criteria for the early-activation stage. Cases in which typical tuberculosis granulomas, macro or microscopic, are seen in any organ, provided no pulmonary involvement, were considered extrapulmonary.

**Results**

**Animals**

Fifteen nonhuman primates from five different colonies in Rio de Janeiro, Brazil, were incorporated into the study. There are 60% (9/15) Old World Monkeys and 40% (6/15) New World Monkeys. The Old World primates belonged to the SCPrim rhesus and cynomolgus colony, seven rhesus monkeys (*Macaca mulatta*) and two cynomolgus monkeys (*Macaca fascicularis*). Among the rhesus monkeys, 71.4% (5/7) were male and 28.5% (2/7) female. The average age of rhesus monkeys was 5 years old. The two cynomolgus monkeys were female, with a mean age of 17 years old. Of the six New World primates, 50% (3/6) were marmosets (*Callithrix* sp.) from the CETAS colony, 33.3% (2/6) were squirrel monkeys (*Saimiri ustus*) from the SCPrim colony, and 16.7% (1/6) was Uta Hick's Bearded Saki (*Chiropotes utahickae*) from the CPRJ colony. Among the marmosets, 66.6% (2/3) were male, and 33.3% (1/3) were female. The marmosets were captured from free-ranging with no previous history. Although it was not possible to determine their exact age, the individuals exhibited sexual maturity. Fifty percent of the squirrel monkeys were male and 50% female (1/2), with an average age of 6.5 year-old. The Uta Hick's Bearded Saki was male and aged 10 years. The sex and age of each nonhuman primate in this study are shown in Table 1.

**Clinical Assessment**

Regardless of disease stage, weight loss, anorexia and lethargy were the main clinical signs observed in the nonhuman primates with tuberculosis in this study. Clinical respiratory signs were observed in 55.5% (5/9) of cases in OWM and 16.7% (1/6) of cases

in NWM. The summary of clinical findings in each case and other relevant clinical considerations are available in Table 1.

**Gross findings**

The changes and severity of macroscopic alterations of all nonhuman primates (NHP) with tuberculosis are available in Table 2. Among the Old World Monkey (OWM) group, 66.7% (6/9) of NHP, all rhesus monkeys, showed severe granulomatous pneumonia with well-demarcated structures, sometimes elevated, firm yellow-white or grayish nodules of different sizes, the typical gross pulmonary granulomas (Fig 1). In all cases, single or coalescing nodules are seen in all pulmonary lobes, but the cranial lobes are most severely affected. The lungs are diffusely firm, enlarged and reddish with multiple consolidations areas. Sometimes, multiple cavitation areas of different sizes are seen on the airways (Fig 1, inset). Ghon's complex presents all six cases with granulomatous pneumonia, with the mediastinal lymph node enlarged in different degrees.

In all cases of tuberculosis in OWM, typical granulomas were seen in at least one organ. The gastrointestinal, lymphatic, musculoskeletal, urinary and nervous systems showed multiple granulomas of variable size and severity and are the most affected systems of multisystemic tuberculosis presentation. In one case (NHP 6), a rhesus monkey presents multiple granulomas of the variable size seen in the brachial plexus nerves (Fig 4). In this group, granulomatous lymphadenitis and granulomatous hepatitis (Fig 2) were seen in 66.7% (6/9) cases, followed by granulomatous splenitis (Fig 3) in 44.44% (4/9) of cases, and granulomatous myositis in 33.3% (3/9) of the cases.

A female rhesus monkey (NHP 2) with no pulmonary involvement showed many granulomas in the trachea, lymph nodes, mesentery, duodenum, pancreas, liver and spleen. In addition to the mesenteric granulomas, this rhesus female presents multifocal variable-sized dark-red cystic masses extending from the uterus to the abdominal wall and serosa of multiple intestine segments, which contained a large amount of dark-red fluid on the cut section. These dark-red cystic masses also are found in multiple abdominal surface organs of a cynomolgus monkey (NHP 9) with considerable enlargement of the mediastinal lymph node and diffuse replacement of tissue architecture by amorphous white areas, multifocal to coalescent yellowish granulomas.

About the New World Monkeys (NWM), the typical pulmonary granulomas were seen in 16.7% (1/6) of the cases, in the Uta Hick's Bearded Saki. The main gross change observed in the NWM was moderate lung increase volume with marked edema in 83.3%

(5/6) of the cases, followed by different degrees and severity of pulmonary consolidation with irregular multifocal red-dark areas 66.7% (4/6) of the cases. In two marmosets (NHPs 10 and 11) has been noted evidence of the hepatic lobular pattern with an increase of the lumen diameter of biliary ducts and gallbladder.

Granulomatous changes were seen in 50% (3/6) of the NWM cases. A marmoset (NHP 12) presented granulomatous myositis, periostitis, and pleuritis (Fig 5). The squirrel monkey (NHP 14) showed granulomatous hepatitis, with many granulomas in the liver parenchyma. In one case, multisystemic granulomatous change was observed in the Uta Hick's Bearded Saki (NHP 15). In this case, the pulmonary granulomas were multifocal and well-delimited structures, sometimes encapsulated, with different sizes in all pulmonary lobes. Some areas of pulmonary lobes creaked when cut (Fig 6). Furthermore, this nonhuman primate presents extrapulmonary spread into the lymph nodes, liver (Fig 7), spleen and kidney (Fig 8).

**Histopathological and immunohistochemical findings**

In this study, 66.7% (6/9) of Old World Monkeys (OWM) and 83.3% (5/6) of New World Monkeys (NWM) showed pulmonary changes at histology. A severe multifocal to coalescent granulomatous pneumonia was seen in all six rhesus monkeys with pulmonary changes and 16.7% (1/6) of NWM tuberculosis cases. In 66.7% (4/6) of NWM tuberculosis cases, different degrees of diffuse foamy interstitial pneumonia were observed with no evidence of necrosis or pulmonary granuloma formation. The pulmonary changes of all nonhuman primates with tuberculosis are summarized in Table 3.

At histology, lung evaluation of the OWM revealed multiple areas with typical caseous granulomas, solid-cellular granuloma (non-necrotizing), and suppurative granuloma in each of the six rhesus monkeys. The caseous granulomas present an acellular center with a large amount of amorphous hypereosinophilic material and pyknotic debris surrounded mainly by epithelioid macrophages, multinucleated giant cells (Langhans and Foreign body giant cells), and a variable number of lymphocytes, neutrophils and rarely foam cells. In the periphery of the caseous granulomas, often were observed solid-cellular granulomas composed of epithelioid macrophages and multinucleated giant cells. In 66.7% (4/6) of cases was observed variable bronchial epithelium ulceration and neutrophils infiltration (NHPs 1, 4, 5, and 7) with the formation of multiple cavities into the major airways (Fig 9).

Besides the lungs, caseous granulomas and solid-cellular granulomas were also found in different severities within multiple organs of these rhesus monkeys. In NHPs 1, 3,

4, 5, 6, and 7 a severe replacement of the lymph node architecture by numerous caseous and solid-cellular granulomas was observed. The liver (Fig 10) (NHPs 1, 3, 4, 5, and 7), spleen (NHPs 3,4,5, and 7), pleura (NHPs 1, 3, 5 and 7), skeletal muscle (NHPs 4, 5 and 6), esophagus (NHP 1), stomach (NHP 4), pancreas (NHP 4), duodenum (NHP 4), kidney (NHP 1), adrenal (NHP 1), joints (NHP 6), brain (NHP 1), and nerves (NHP 6) also presented multiple granulomas in different severities.

In 50% (3/6) of OWM cases with granulomatous pneumonia, it was possible to visualize straight or slightly curved bacilli, with 1 to 5 µm long and 0.2 to 0.8 µm wide into the areas of necrosis or inside the cytoplasm of macrophages, epithelioid macrophages and multinucleated giant cells using the Zieh-Neelsen technique (Fig 11). Immunohistochemistry with anti-*Mycobacterium tuberculosis* showed intact and degenerated bacilli amidst the necrotic areas and intracytoplasmic in multinucleated giant cells, macrophages and epithelioid macrophages, all six cases of granulomatous pneumonia analyzed (Fig 12).

A rhesus monkey (NHP 2) with no pulmonary changes showed multifocal to coalescent typical caseous granulomas in the trachea, lymph nodes, mesentery, duodenum, pancreas, liver and spleen. The dark-red cystic masses of the abdomen wall (Fig 13) and mesenteric nodules at the histological evaluation, in addition to the tuberculosis granulomas, a moderately cellular proliferation composed of columnar and ciliated endometrial cells with a moderate amount of clear eosinophilic cytoplasm and round nuclei. Multifocally, there are areas with a moderate amount of free red blood cells (hemorrhage), accompanied by macrophages with abundant intracytoplasmatic brown pigments (hemosiderophages). These findings are compatible with endometriosis (Fig. 14).

A cynomolgus monkey (NHP 9) without any pulmonary impairment also presented endometriosis. Moreover, this cynomolgus presents a multifocal to coalescent granulomatous lymphadenitis with intense replacement of the typical architecture of the lymph node by severe caseous granulomas formation. In a rhesus monkey (NHP 8), histological changes were observed just in the cardiac ganglia. The neuronal cell bodies of the ganglia are expanded by a few multinucleated giant cells of foreign body type. Adjacent to that areas and expanding the epicardium were seen well-demarcated multifocal areas with necrotic center and dystrophic mineralization.

Out of three New World Monkeys that present histopathological pulmonary changes, two (66.7%) marmosets and one squirrel monkey (NHP 13) presented different degrees of the proliferation of foamy macrophages that thicken the alveolar septa and

infiltrate the air space (Fig. 15). These macrophages exhibit ample foamy cytoplasm and often concentric and lateralized nucleus. Although few giant cells were seen in one marmoset (NHP 10), no evidence of necrosis or granuloma formation was noted in lung sections or any other organ on this NWM.

A squirrel monkey (NHP 14) showed multifocal granulomatous hepatitis with a large number of solid-cellular granulomas and few caseous granulomas surrounded mainly by some epithelioid macrophages, multinucleated giant cells, and lymphocytes. The marmoset (NHP 12) presents a granulomatous pleuritis with a large caseous granuloma in the intercostal muscle adjacent to the pleura. A mild diffuse interstitial pneumonia with the predominance of lymphocytes was observed in this marmoset. That was the only case with pneumonia in this group.

A significant number of lymphocytes and eosinophils are seen around the ectatic ducts, and several fibroblastic proliferation and multifocal areas increased in the number of bile ducts (biliary hyperplasia). Two marmosets of the early activation stage group (NHPs 10 and 11) showed a severe lymphocytic and eosinophilic proliferative cholangitis at histology, with multiple free eggs and structures compatible with adult trematodes inside and obstructing partially the lumen of biliary ducts, that were often ecstatic. These adult trematodes have prominent oral and ventral suckers, a thin outer integument covering a somatic musculature, a parenchymatous matrix, a digestive tract with paired ceca, vitellaria, and a uterus with numerous, oval, yellow-brown singly operculated, thick eggs with miracidia.

In the only case of granulomatous multifocal to coalescent bronchopneumonia in the NWM, the Uta Hick's Bearded Saki showed a large number of well-demarcated and encapsulated mineralized granulomas with variable sizes in the pulmonary parenchyma (Fig 16). These granulomas are often surrounded by a significant number of foamy macrophages with abundant cytoplasm, epithelioid macrophages, lymphocytes and a few multinucleated giant cells and neutrophils. Adjacent to those areas, the alveolar space showed a severe amount of amorphous eosinophilic material (edema). Sometimes, multifocal fibroblastic proliferation in the alveolar wall is seen in the lung sections. A great number of foamy macrophages infiltrated multifocally, the airways and the bronchial and bronchiolar lumen.

Through the evaluation using the Ziehl-Neelsen technique, a few bacilli, predominantly intracellular in foamy cells of the marmosets and extracellularly in the necrosis areas of the Uta Hick’s Bearded Saki (Fig 17), were visualized in 83.3% (5/6) of the

cases. There was evidence of intracellular bacilli in all cases, mainly in the airways and intrabronchial foamy macrophages (Fig 18). The Ziehl-Neelsen and immunohistochemical results of each case of NHP with tuberculosis are available in Table 4.

**Molecular findings**

The molecular analysis was performed in fourteen of fifteen cases of the nonhuman primates in this study. In the molecular detection with oligonucleotides INS1 and INS2, 64.28%(9/14) were positive, while with oligonucleotides TB11 and TB12, 14.28%(2/14) were positive and submitted to sequencing, confirming the diagnosis for CMTB. The GeneXpert® system was able to detect the presence of tuberculosis complex species DNA in all analyzed samples. Additionally, all samples were identified as sensitive to the antibiotic rifampicin, amplifying the genes rpoB1, rpoB2, rpoB3, and rpoB4. The molecular results of each case are available in Table 4.

**Bacterial culture**

The bacterial culture was performed in six of fifteen cases of the nonhuman primates in this study. In all cases was observed the growth of *Mycobacterium tuberculosis* complex bacteria. The bacterial culture results of each case are available in Table 4.

**Tuberculosis stage classification**

Based on clinical, morphological, immunohistochemical, and, when possible molecular and bacteriological results, the nonhuman primates are classified in different tuberculosis stages (Table 5). Out of nine Old World Monkeys (OWM), 66.7% six cases were classified as active-chronic tuberculosis stage. Among these primates, typical pulmonary granulomas were seen in all cases in this group and clinical respiratory signs in 83.3% (5/6) of cases. In 33.3% (3/9) OWM cases, the stage was classified as extrapulmonary due to the absence of gross or histologic granulomatous pneumonia. Extrapulmonary tuberculosis was seen in two cynomolgus monkeys (*M. fascicularis*) and one rhesus monkey (*M. mulatta*). In this group, respiratory signs were observed in 33.3% (1/3) of the cases.

In the New World Monkeys (NWM), 50% (3/6) of the cases were classified as the early-activation stage, 33.3% (2/6) as extrapulmonary stage and 16.7% (1/6) as latent-reactivation tuberculosis stage. In the early-activate group, 66.7% (2/3) of the primates were marmosets (*Callithrix* sp.), and 33.3% (1/3) were squirrel monkeys (*S. ustus*). In none of the early activation cases, pulmonary granulomas were observed, and only the squirrel monkey showed clinical respiratory signs. The extrapulmonary stage group was composed of one marmoset (*Callithrix* sp.) and one squirrel monkey (*S. ustus*). No clinical respiratory signs

were observed in the NWM of the early-activate stage. The latent-reactivation stage was observed in the Uta Hick's Bearded Saki (*C. utahickae*). This primate had typical latent pulmonary granulomas, and no clinical respiratory signs were observed.

**Discussion**

Prior studies with experimental infections of *Mycobacterium tuberculosis* Complex (MTBC) in nonhuman primates (NHPs) have described the morphological heterogeneity of tuberculosis (Capuano et al., 2003; Lin et al., 2014; Cadena et al., 2017). In humans, pulmonary granuloma formation is considered the pathological hallmark of tuberculosis (Wadee and Wadee, 2021). In NHPs infected with MTBC, a large spectrum of lesions can be seen within and between monkeys of the same stage classification as well described in humans (Capuano et al, 2003; Lin et al, 2009; Flynn and Klein 2011). Although the typical tuberculosis presentation was observed in some cases, the absence of pulmonary granuloma did not exclude the tuberculosis occurrence of extrapulmonary impairment or early pulmonary changes in NHPs of the Old and New World naturally infected with MTBC. We believe that the main differences between the tuberculosis pathology in NHPs are due to the stage disease presentation.

We observed predominance of the chronic-active tuberculosis stage in OWM, especially in rhesus monkeys (*Macaca mulatta*), compared to the cynomolgus monkey (*M. fascicularis*). Presumably, this fact occurs because the rhesus macaques are more susceptible to the *M. tuberculosis* infection than cynomolgus macaques (Langermans et al., 2001; Maiello et al., 2018). This fact may have favored the activation stage of the disease in rhesus monkeys. In this natural occurrence of chronic-active tuberculosis in rhesus monkeys, all nonhuman primates present changes compatible with descriptions of experimental infectious tuberculosis in this species (Zhang et al., 2011; Zhang et al., 2014). In our study, all rhesus monkeys of chronic-active tuberculosis present a full spectrum of histopathological changes with different granulomas types in the same individual, as well seen in humans (Capuano et al., 2003).

Extrapulmonary tuberculosis refers to TB involving potentially any organ other than the lungs, and reports of this presentation in macaques are scarce in the literature (Stockinger et al., 2011; Lee 2015). According to Capuano et al., (2003), the extrapulmonary spread was variable among nonhuman primates, and when the disease is advanced, the nonhuman primates can exhibit extrapulmonary dissemination. The macroscopic and histopathological granulomatous lesions in several organs of a *M. mulatta*, lymph nodes and heart of *M.*

*Fascicularis*, liver of the *S. ustus*, skeletal muscle and parietal pleura of *Callithrix* sp. show the plasticity of MTBC to cause different changes in multiple organs in infectious individuals even when pulmonary changes were absent. Due to these findings, we suspect that the systemic involvement has resulted from the hematogenous spreading of the MTBC bacilli after the initial infection.

Hematogenous dissemination within organs can occur in tuberculosis infectious. Despite wide dissemination of *Mtb* during primary infection, the majority of infected, but otherwise healthy, individuals resolve these lesions without becoming symptomatic (Sakamoto, 2012). Nonhuman primates exposure to *M. tuberculosis* results in a wide range of outcomes (Scanga and Flynn, 2014). Unlike the granulomatous changes in many organs, our extrapulmonary results suggest a possible pulmonary resolution due to an absence of clinical respiratory signs in the mostly nonhuman primates with this stage presentation. None factor was associated as a cause of the high prevalence of extrapulmonary tuberculosis in nonhuman primates of this study. The concomitance of endometriosis and abdominoperitoneal tuberculosis were described in humans, especially in infertile women (Sharma et al., 2017). These condition can co-exist in nonhuman primates without previously described mechanism. We suggest further studies to determine the pathogenesis of concomitance.

The formation of foam cells is a manifestation of maladaptive responses occurring during many inflammatory conditions (Hotamisligil, 2017). In humans, there is a wide literature detailing the presence of foamy macrophages in tuberculosis granulomas (Hunter et al., 2007; Peyron et al., 2008). Although the occurrence of foam cells is typically associated with necrotic granulomas (Peyron et al., 2008), the absence of necrosis, granulomatous changes or interstitial fibrosis in the early-activation tuberculosis stage can indicate that foamy macrophages seemed to be a key component in the initial activation pathogenesis of tuberculosis in nonhuman primates.

The lesions of early pulmonary tuberculosis can develop along several pathways (Hunter, 2011). In the early-activation stage, we observed diffuse interstitial foamy pneumonia. Although lipidic pneumonia was associated with post-primary tuberculosis (Hunter et al., 2007), no evidence of endobronchial tuberculosis producing bronchial obstruction, necrosis, or cavitation was found in the NHPs lungs with early-activation stage in this study. In one marmoset of the early-activation lung stage, we observed a great number of interstitial foamy macrophages and few multinucleated giant cells without necrosis. That

can be an initial fact associated with tuberculosis granuloma formation. Presumably, the initial granuloma formation mechanism, especially in NWM needs to be better elucidated in further studies.

Studies have proposed that intracellular macrophage bacilli overproduce Mtb lipids; these lipids consolidate in the internal vesicles in the multivesicular body and are subsequently exocytosed into the extracellular medium (Beatty et al., 2000; Beatty et al., 2001). It was demonstrated that foam cell formation is induced explicitly by oxygenated forms of mycolic acid, such as oxygenated ketomycolic and hydroxyl-mycolic acids synthesized by pathogenic mycobacterial species such *Mtb* (Peyron et al., 2008; Russel et al., 2009). In our study, the presence of bacilli in the foamy macrophages was confirmed through de acid-fast stain and immunohistochemistry technique.

As in humans, tuberculosis in nonhuman primates can be stably maintained, spontaneously reactivate, or reactivate in response to some immune suppression (Lin et al., 2009; Lin and Flynn, 2010). Did not possible to determine an immunosuppression factor associated with the latent-reactivation stage in Uta Hick's Bearded Saki in this study, and that appears to have spontaneously reactivated a latent infection. We observed an intense intracytoplasmatic immunolabelling to anti-*Mycobacterium tuberculosis* on foamy macrophages of the latent-reactivation stage. This fact also indicates the foam cells formation due to an active *M. tuberculosis* replication. Besides the necrotizing granulomas, this monkey showed nonnecrotizing granulomas in the lung tissues that have not been observed in latent infection in nonhuman primates (Lin et al., 2009).

This study shows that clinical respiratory signs should not be considered solely for the clinical suspicion of tuberculosis in nonhuman primates. Coughing is infrequent in nonhuman primates with tuberculosis but may occur (Mansfield and Fox, 2019), like in the three cases of rhesus monkey and one squirrel monkey (*S. ustus*) from this survey. In our study, weight loss, lethargy and anorexia were the most frequent clinical signs in nonhuman primates with tuberculosis in according to described by Simmons and Gibson (2012) and Via et al. (2003).

The tuberculosis diagnosis in the nonhuman primates in this study was made based on the association of pathological, immunohistochemical, molecular, and when possible bacteriological culture findings. The presence of mycobacterial antigens and tissue morphology can be evaluated together using the immunohistochemistry technique (Karimi et al., 2014). Due to the high sensitivity of this technique compared to the acid-fast stain, we

recommend for routine diagnostic its use even in cases without typical tuberculosis microscopic changes. The molecular analysis presents high sensitivity and specificity for the discrimination of microorganisms belonging to the *M. tuberculosis* Complex (Neshani et al., 2018). Although ampicillin resistance was not diagnosed in any case in the present study, we suggest using GeneXpert® as a surveillance tool for bacterial resistance to antibiotics.

All nonhuman primates of this study were naturally infected with *Mycobacterium tuberculosis* Complex, although reports stated that the Old World Monkeys are more susceptible than New World Monkeys (Brack, 1987; Montali, 2001; Blanchard and Russell-Lodrigue 2012). No predisposing factors were observed in Old World nonhuman primates compared to New World primates. The occurrence of TB in free-ranging nonhuman primates is considered uncommon (Rocha et al, 2011). We believe that all nonhuman primates under human care with direct contact with humans are susceptible to *M. tuberculosis* infection. Due to the natural occurrence of MTBC infection in this study was not possible to determine the route, strain, infectious dose and time of evolution of the tuberculosis infection.

Complete and specific exams for the *antemortem* tuberculosis diagnosis must be carried out constantly in the nonhuman primates colonies, even when clinical respiratory signs are not evident. Due to the possible retransmission from monkeys to man (Mätz-Rensing et al, 2015), the occurrence of tuberculosis in nonhuman primates offers a potential health risk for other staff members. Owing to the inherent risk a nonhuman primate infected with tuberculosis poses to the colony and staff, it is recommended that monkeys infected with or suspected of being infected with tuberculosis be euthanized (National Research Council, 2003; Bushmitz et al., 2009).

In conclusion, we observe a wide spectrum of morphological features comparing the nonhuman primates diagnosed with different tuberculosis stages. Classical granulomatous pneumonia was observed in the chronic-active and latent-reactivation stages but not in the extrapulmonary and early-activation stages. The early-activation stage in primates is characterized by foamy interstitial pneumonia without typical tuberculosis granulomas. Due to this fact, tuberculosis should be included as a cause of foamy interstitial pneumonia in nonhuman primates, especially in the New World species. Experimental studies need to be realized to elucidate the exact role of the foamy macrophage in the early stage of tuberculosis. We recommend the addition of immunohistochemistry as a diagnostic tool of tuberculosis even when typical macroscopic or histologic changes are not observed.

**Acknowledgments**

We are thankful to the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) for the support of our study. We are thankfull to Edson Moleta Colodel for the contributions and revision of the draft paper.

**Conflict of Interest Statement**

The authors declared no potential conflicts of interest concerning this article's research, authorship, and publication.

**References**

Balansard, I., Cleverley, L., Cutler, K.L., Spångberg, M.G., Thibault-Duprey, K. and Langermans, J.A., 2019. Revised recommendations for health monitoring of non-human primate colonies (2018): FELASA Working Group Report. Laboratory animals, 53(5), pp.429-446.

Beatty, W.L., Rhoades, E.R., Ullrich, H.J., Chatterjee, D., Heuser, J.E. and Russell, D.G., 2000. Trafficking and release of mycobacterial lipids from infected macrophages. Traffic, 1(3), pp.235-247.

Beatty, W.L., Ullrich, H.J. and Russell, D.G., 2001. Mycobacterial surface moieties are released from infected macrophages by a constitutive exocytic event. European journal of cell biology, 80(1), pp.31-40.

Blanchard, J.L., Russell-Lodrigue, K.E. 2012. Biosafety in laboratories using nonhuman primates. In Nonhuman Primates in Biomedical Research, Vol. 1: Biology and Management, 2nd ed., Chapter 18, C. R. Abee, K. Mansfield, S. Tardif, and T. Morris (eds.). Waltham, MA: Academic Press.

Brasil. Ministério da Ciência, Tecnologia e Inovação/Conselho Nacional de Controle de Experimentação Animal (CONCEA). Resolução Normativa Nº 28 de 13 de Novembro de 2015. Diário Oficial da União. Brasília, D.F., 16 nov. 2015. Disponível em: http://www.in.gov.br/materia/-/asset_publisher/Kujrw0TZC2Mb/content/id/33322952/do1-2015-11-16-resolucao-normativa-n-28-de-13-de-novembro-de-2015-33322892. Acesso em: 03 abr. 2020.

Brasil. Ministério da Saúde. Secretaria de Vigilância em Saúde. Departamento de Doenças de Condições Crônicas e Infecções Sexualmente Transmissíveis – DCCI. Boletim Epidemiológico Especial, Número Especial, Tuberculose, 2020.

Bushmitz, M., Lecu, A., Verreck, F., Preussing, E., Rensing, S., Mätz-Rensing, K. and EPV-Tuberculosis Working Group on Non-human Primate Health, 2009. Guidelines for the

prevention and control of tuberculosis in non-human primates: Recommendations of the European Primate Veterinary Association Working Group on Tuberculosis. Journal of Medical Primatology, 38(1), pp.59-69.

Cadena, A.M., Fortune, S.M. and Flynn, J.L., 2017. Heterogeneity in tuberculosis. Nature Reviews Immunology, 17(11), pp.691-702.

Capuano III, S.V., Croix, D.A., Pawar, S., Zinovik, A., Myers, A., Lin, P.L., Bissel, S., Fuhrman, C., Klein, E. and Flynn, J.L., 2003. Experimental *Mycobacterium tuberculosis* infection of cynomolgus macaques closely resembles the various manifestations of human M. tuberculosis infection. Infection and immunity, 71(10), pp.5831-5844.

Clarke, G.L., 1968. The relationship of hypersensitivity to shedding of Mycobacterium tuberculosis in experimentally infected Macaca mulatta. American Review of Respiratory Disease, 98(3), pp.416-423.

da Silva, D.A., Rêgo, A.M., Ferreira, N.V., de Andrade, M.A., Campelo, A.R., Caldas, P.C.S., Pereira, M.A.S., Redner, P., de Pina, L.C., Resende, F.C. and Pissinatti, T.A., 2017. Detection of mycobacterial infection in non-human primates using the Xpert MTB/RIF molecular assay. Tuberculosis, 107, pp.59-62.

Flynn JL, Klein E 2011. Pulmonary tuberculosis in monkeys. In A color atlas of comparative pulmonary tuberculosis histopathology (ed. Leong VDJ, Dick T), pp. 83–106 CRC, Boca Raton, FL.

Foreman, T.W., Mehra, S., Lackner, A.A. and Kaushal, D., 2017. Translational research in the nonhuman primate model of tuberculosis. ILAR journal, 58(2), pp.151-159.

Ghodbane, R. and Drancourt, M., 2013. Non-human sources of Mycobacterium tuberculosis. Tuberculosis, 93(6), pp.589-595.

Hotamisligil, G.S., 2017. Foundations of immunometabolism and implications for metabolic health and disease. Immunity, 47(3), pp.406-420.

Hunter, R.L., 2011. Pathology of post primary tuberculosis of the lung: an illustrated critical review. Tuberculosis, 91(6), pp.497-509.

Hunter, R.L., Jagannath, C. and Actor, J.K., 2007. Pathology of postprimary tuberculosis in humans and mice: contradiction of long-held beliefs. Tuberculosis, 87(4), pp.267-278.

Karimi, S., Shamaei, M., Pourabdollah, M., Sadr, M., Karbasi, M., Kiani, A. and Bahadori, M., 2014. Histopathological findings in immunohistological staining of the granulomatous tissue reaction associated with tuberculosis. Tuberculosis research and treatment, 2014.

Kanabalan, R.D., Lee, L.J., Lee, T.Y., Chong, P.P., Hassan, L., Ismail, R. and Chin, V.K., 2021. Human tuberculosis and Mycobacterium tuberculosis complex: A review on genetic diversity, pathogenesis and omics approaches in host biomarkers discovery. Microbiological research, p.126674.

Kaushal, D., Mehra, S., Didier, P.J. and Lackner, A.A., 2012. The non-human primate model of tuberculosis. Journal of medical primatology, 41(3), pp.191-201.

Kudoh, S. and Kudoh, T., 1974. A simple technique for culturing tubercle bacilli. Bulletin of the World Health Organization, 51(1), p.71.

Lenaerts, A., Barry III, C.E. and Dartois, V., 2015. Heterogeneity in tuberculosis pathology, microenvironments and therapeutic responses. Immunological reviews, 264(1), pp.288-307.

Langermans, J.A., Andersen, P., van Soolingen, D., Vervenne, R.A., Frost, P.A., van der Laan, T., van Pinxteren, L.A., van den Hombergh, J., Kroon, S., Peekel, I. and Florquin, S., 2001. Divergent effect of bacillus Calmette–Guerin (BCG) vaccination on *Mycobacterium tuberculosis* infection in highly related macaque species: Implications for primate models in tuberculosis vaccine research. Proceedings of the National Academy of Sciences, 98(20), pp.11497-11502.

Lee, J.Y., 2015. Diagnosis and treatment of extrapulmonary tuberculosis. Tuberculosis and respiratory diseases, 78(2), pp.47-55.

Lin, P.L., Rodgers, M., Smith, L.K., Bigbee, M., Myers, A., Bigbee, C., Chiosea, I., Capuano, S.V., Fuhrman, C., Klein, E. and Flynn, J.L., 2009. Quantitative comparison of active and latent tuberculosis in the cynomolgus macaque model. Infection and immunity, 77(10), pp.4631-4642.

Lin, P.L. and Flynn, J.L., 2010. Understanding latent tuberculosis: a moving target. The Journal of Immunology, 185(1), pp.15-22.

Lin, P.L., Ford, C.B., Coleman, M.T., Myers, A.J., Gawande, R., Ioerger, T., Sacchettini, J., Fortune, S.M. and Flynn, J.L., 2014. Sterilization of granulomas is common in active and latent tuberculosis despite within-host variability in bacterial killing. Nature medicine, 20(1), pp.75-79.

Maiello, P., DiFazio, R.M., Cadena, A.M., Rodgers, M.A., Lin, P.L., Scanga, C.A. and Flynn, J.L., 2018. Rhesus macaques are more susceptible to progressive tuberculosis than cynomolgus macaques: a quantitative comparison. Infection and immunity, 86(2), pp.e00505-17.

Mätz-Rensing, K.; Lowenstine, L.J. New World and Old World monkeys. In: Terio KA, McAloose D, St. Leger J (eds) Pathology of Wildlife and Zoo Animals. Academic Press, San Diego, p. 343– 374, 2018.

Mehra, S., Alvarez, X., Didier, P.J., Doyle, L.A., Blanchard, J.L., Lackner, A.A. and Kaushal, D., 2013. Granuloma correlates of protection against tuberculosis and mechanisms of immune modulation by *Mycobacterium tuberculosis*. The Journal of infectious diseases, 207(7), pp.1115-1127.

National Research Council. 2003. Occupational Health and Safety in the Care and Use of Nonhuman Primates. Washington, DC: National Academies Press.

Neshani, A., Kakhki, R.K., Sankian, M., Zare, H., Chichaklu, A.H., Sayyadi, M. and Ghazvini, K., 2018. Modified genome comparison method: a new approach for identification of specific targets in molecular diagnostic tests using *Mycobacterium tuberculosis* complex as an example. BMC infectious diseases, 18(1), pp.1-9.

Peyron, P., Vaubourgeix, J., Poquet, Y., Levillain, F., Botanch, C., Bardou, F., Daffé, M., Emile, J.F., Marchou, B., Cardona, P.J. and De Chastellier, C., 2008. Foamy macrophages from tuberculous patients' granulomas constitute a nutrient-rich reservoir for *M. tuberculosis* persistence. PLoS pathogens, 4(11), p.e1000204.

Okada, M., Kita, Y., Nakajima, T., Kanamaru, N., Hashimoto, S., Nagasawa, T., Kaneda, Y., Yoshida, S., Nishida, Y., Fukamizu, R. and Tsunai, Y., 2007. Evaluation of a novel vaccine (HVJ–liposome/HSP65 DNA+ IL-12 DNA) against tuberculosis using the cynomolgus monkey model of TB. Vaccine, 25(16), pp.2990-2993.

Reed, S.G., Coler, R.N., Dalemans, W., Tan, E.V., Cruz, E.C.D., Basaraba, R.J., Orme, I.M., Skeiky, Y.A., Alderson, M.R., Cowgill, K.D. and Prieels, J.P., 2009. Defined tuberculosis vaccine, Mtb72F/AS02A, evidence of protection in cynomolgus monkeys. Proceedings of the National Academy of Sciences, 106(7), pp.2301-2306.

Rocha, V.C.M., Ikuta, C.Y., Gomes, M.S., Quaglia, F., Matushima, E.R. and Neto, J.S.F., 2011. Isolation of *Mycobacterium tuberculosis* from captive *Ateles paniscus*. Vector-Borne and Zoonotic Diseases, 11(5), pp.593-594.

Russell, D.G., Cardona, P.J., Kim, M.J., Allain, S. and Altare, F., 2009. Foamy macrophages and the progression of the human tuberculosis granuloma. Nature immunology, 10(9), pp.943-948.

Sambrook, J. Russel, D.W. 2001. Molecular Cloning: A laboratory Manual. 2ed. New York, Cold Spring Harbor Laboratory Press. 2001.

Scanga, C.A. and Flynn, J.L., 2014. Modeling tuberculosis in nonhuman primates. Cold Spring Harbor perspectives in medicine, 4(12), p.a018564.

Schmidt, L.H., 1956. Some observations on the utility of simian pulmonary tuberculosis in defining the therapeutic potentialities of isoniazid. American Review of Tuberculosis and Pulmonary Diseases, 74(2-2), pp.138-152.

Sharma, J.B., Goyal, M., Kumar, S., Roy, K.K., Sharma, E. and Arora, R., 2017. Concomitant female genital tuberculosis and endometriosis. Indian Journal of Tuberculosis, 64(3), pp.173-177.

Shi, S.R., Datar, R., Liu, C., Wu, L., Zhang, Z., Cote, R.J. and Taylor, C.R., 2004. DNA extraction from archival formalin-fixed, paraffin-embedded tissues: heat-induced retrieval in alkaline solution. Histochemistry and cell biology, 122(3), pp.211-218.

Simons, J., Gibson, S.V. 2012. Bacterial and mycotic diseases. In Nonhuman Primates in Biomedical Research, 2th ed, Academic Press. p.113

Stockinger, D.E., Roellich, K.M., Vogel, K.W., Eiffert, K.L., Torrence, A.E., Prentice, J.L., Stephens, K.G., Wallis, C.K., Hotchkiss, C.E. and Murnane, R.D., 2011. Primary hepatic *Mycobacterium tuberculosis* complex infection with terminal dissemination in a pig-tailed macaque (*Macaca nemestrina*). Journal of the American Association for Laboratory Animal Science, 50(2), pp.258-262.

Telenti, A., Marchesi, F., Balz, M., Bally, F., Böttger, E.C. and Bodmer, T., 1993. Rapid identification of mycobacteria to the species level by polymerase chain reaction and restriction enzyme analysis. Journal of clinical microbiology, 31(2), pp.175-178.

Wadee, R. and Wadee, A.A., 2021. The Pathology of Lymphocytes, Histiocytes, and Immune Mechanisms in *Mycobacterium tuberculosis* Granulomas. The American Journal of Tropical Medicine and Hygiene, 104(5), pp.1796-1802.

Walsh, G.P., Tan, E.V., Cruz, E.C.D., Abalos, R.M., Villahermosa, L.G., Young, L.J., Cellona, R.V., Narareno, J.B. and Horwitz, M.A., 1996. The Philippine cynomolgus monkey (*Macaca fasicularis*) provides a new nonhuman primate model of tuberculosis that resembles human disease. Nature medicine, 2(4), pp.430-436.

Warit, S., Billamas, P., Makhao, N., Jaitrong, S., Juthayothin, T., Yindeeyoungyeon, W., Dokladda, K., Smittipat, N., Kemthong, T., Meesawat, S. and Kongsombat, N., 2020. Detection of tuberculosis in cynomolgus macaques (*Macaca fascicularis*) using a supplementary Monkey Interferon Gamma Releasing Assay (mIGRA). Scientific reports, 10(1), pp.1-11..

World Health Organization. Global Tuberculosis Report. Executive Summary, 2019
World Health Organization. Tuberculosis: Key facts Geneva, Switzerland. 2020a
World Health Organization. 2020b Global Tuberculosis Report. The World Health Organization (2020). Available at: https://www.who.int/tb/ publications/global_report/en/
Zhang, J., Ye, Y.Q., Wang, Y., Mo, P.Z., Xian, Q.Y., Rao, Y., Bao, R., Dai, M., Liu, J.Y., Guo, M. and Wang, X., 2011. *M. tuberculosis* H37Rv infection of Chinese rhesus macaques. Journal of Neuroimmune Pharmacology, 6(3), pp.362-370..
Zhang, J., Xian, Q., Guo, M., Huang, Z., Rao, Y., Wang, Y., Wang, X., Bao, R., Evans, T.G., Hokey, D. and Sizemore, D., 2014. *Mycobacterium tuberculosis* Erdman infection of rhesus macaques of Chinese origin. Tuberculosis, 94(6), pp.634-643.

# Tables

## Table 1 – Clinical evaluation of New and Old World Monkeys with tuberculosis

| | NHP | Species | Sex | Age* | General Parameters | | | Respiratory Parameters | | | | Other considerations |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | Anorexia | Weight Loss | Lethargy | Cough | Sneeze | Dyspnea | Tachypnea | |
| Old World Monkeys | 1 | *Macaca mulatta* | M | 4 | ++ | ++ | +++ | ++ | - | + | + | - |
| | 2 | *Macaca mulatta* | F | 9 | + | ++ | +++ | - | - | +++ | +++ | - |
| | 3 | *Macaca mulatta* | F | 9 | - | - | - | - | - | - | - | Unknown previous clinical history. |
| | 4 | *Macaca mulata* | M | 3 | ++ | +++ | +++ | +++ | - | +++ | +++ | - |
| | 5 | *Macaca mulatta* | M | 4 | ++ | +++ | ++++ | +++ | - | - | - | - |
| | 6 | *Macaca mulatta* | M | 9 | ++ | +++ | +++ | - | - | ++ | ++ | Severe lameness in the left pelvic limb |
| | 7 | *Macaca mulatta* | M | 3 | - | - | - | - | - | - | - | Unknown previous clinical history. |
| | 8 | *Macaca fascicularis* | F | 20 | - | - | - | - | - | - | - | Diarrhea and severe dehydration |
| | 9 | *Macaca fascicularis* | F | 14 | - | - | - | - | - | - | - | Euthanasia due to endometriosis |
| New World Monkeys | 10 | *Callithrix* spp. | F | Adult | +++ | +++ | +++ | - | - | - | - | Proliferative cholangitis due to adults trematodes. |
| | 11 | *Callithrix* spp. | M | Adult | +++ | +++ | +++ | - | - | - | - | Proliferative cholangitis due to adults trematodes |
| | 12 | *Callithrix* spp. | M | Adult | ++ | +++ | +++ | - | - | - | - | - |
| | 13 | *Saimiri ustus* | M | 10 | +++ | +++ | +++ | +++ | +++ | +++ | +++ | - |
| | 14 | *Saimiri ustus* | F | 3 | - | - | - | - | - | - | - | Unknown previous clinical history |
| | 15 | *Chiropotes utahickae* | M | 10 | +++ | +++ | +++ | - | - | - | - | - |

**Table 2 – Gross findings of tuberculosis in New and Old World Monkeys**

| Gross finding | Old World Monkeys | | New World Monkeys | | |
|---|---|---|---|---|---|
| | ***Macaca mulatta* (n=7)** | ***Macaca fascicularis* (n=2)** | ***Callithrix* spp. (n=3)** | ***Saimiri ustus* (n=2)** | ***Chiropotes utahickae* (n=1)** |
| | Amount/Severity | Amount/Severity | Amount/Severity | Amount/Severity | Amount/Severity |
| Lung | | | | | |
| Edema | 6/ +++ | 2/ + | 3/ +++ | 1/ ++ | 1/ +++ |
| Consolidation | 6/ +++ | - | 2/ + | 1/++ | 1/ +++ |
| Granulomatous pneumonia | | | | | |
| Cranial lobes | 6/ +++ | - | - | - | 1/ +++ |
| Caudal lobes | 6/ ++ | - | - | - | 1/++ |
| Granulomatous pleuritis | 4/ +++ | - | 1/ +++ | - | - |
| Granulomatous lymphadenitis | | | | | |
| Mediastinal lymph node | 6/ +++ | - | - | - | 1/ +++ |
| Mesenteric lymph node | 3/ +++ | - | - | - | 1/ +++ |
| Others lymph nodes | 5/ ++ | 1/ +++ | - | - | 1/ +++ |
| Granulomatous tracheitis | 1/ +++ | - | - | - | 1/ +++ |
| Granulomatous esophagitis | 1/ +++ | - | - | - | - |
| Granulomatous gastritis | 1/ +++ | - | - | - | - |
| Granulomatous pancreatitis | 2/ +++ | - | - | - | - |
| Granulomatous duodenitis | 1/ +++ | - | - | - | - |
| Granulomatous hepatitis | 6/ +++ | - | - | 1 / ++ | 1/ +++ |
| Granulomatous splenitis | 4/ +++ | - | - | - | - |
| Granulomatous nephritis | 1/ +++ | - | - | - | - |
| Granulomatous epicarditis | - | 1/ ++ | - | - | 1/ +++ |
| Granulomatous encephalitis | 1/ +++ | - | - | - | - |

| | | | | | |
|---|---|---|---|---|---|
| Granulomatous neuritis | 1/ +++ | - | - | - | - |
| Granulomatous arthritis | 1/ +++ | - | - | - | - |
| Granulomatous myositis | 3/ +++ | - | 1/++ | - | - |
| Others | | | | | |
| Endometriosis | 1/ +++ | 1/ +++ | - | - | - |
| Chronic Proliferative Cholangitis by trematoda | | | | | |

(-) Absent; (+) Mild; (++) Moderate; (+++) Severe.

**Table 3– Pulmonary histopathological findings of tuberculosis in New and Old World Monkeys**

| | NHP | Species | Distribuition of pneumonia / severity | Granulomas | | | | Inflammatory cells | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Caseous necrosis | Mineralization | Fibrous capsule | Cavitation | Giant cells | Epithelioid Macrophages | Foamy Macrophages | Lymphocytes | Neutrophils |
| Old World Monkeys | 1 | *Macaca mulatta* | MTC/+++ | +++ | - | + | + | + | ++ | + | ++ | +++ |
| | 2 | *Macaca mulatta* | - | - | - | - | - | - | - | - | - | - |
| | 3 | *Macaca mulatta* | MTC/+++ | +++ | - | +++ | - | ++ | ++ | + | ++ | - |
| | 4 | *Macaca mulatta* | MTC/+++ | +++ | - | +++ | +++ | +++ | +++ | - | +++ | + |
| | 5 | *Macaca mulatta* | MTC/+++ | +++ | - | ++ | + | + | ++ | - | +++ | ++ |
| | 6 | *Macaca mulatta* | MTC/+++ | +++ | - | ++ | - | + | ++ | + | +++ | + |
| | 7 | *Macaca mulatta* | MTC/+++ | +++ | - | ++ | ++ | +++ | ++ | - | ++ | +++ |
| | 8 | *Macaca fascicularis* | - | - | - | - | - | - | - | - | - | - |
| | 9 | *Macaca fascicularis* | - | - | - | - | - | - | - | - | - | - |
| New World Monkeys | 10 | *Callithrix* spp. | ID/+++ | - | - | - | - | + | - | +++ | +++ | -- |
| | 11 | *Callithrix* spp. | ID/+++ | - | - | - | - | - | - | ++ | ++ | - |
| | 12 | *Callithrix* spp. | ID/+ | - | - | - | - | - | - | - | +++ | - |
| | 13 | *Saimiri ustus* | ID/++ | - | - | - | - | - | -- | ++ | + | - |
| | 14 | *Saimiri ustus* | - | - | - | - | - | - | - | - | - | - |
| | 15 | *Chiropotes utahickae* | MTC/+++ | +++ | +++ | + | - | + | + | +++ | +++ | + |

Mtc= Multifocal to coalescent; ID= Interstitial difuse; (-) Absent; (+) Mild; (++) Moderate; (+++) Severe.

**Table 4 – Methods applied to diagnostic of tuberculosis in New and Old World Monkeys**

| | | | Evaluation of bacilli in histology sections | | | Molecular analysis | | | MTBC[#] culture | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Ziehl-Nielsen | | Anti-*Mtb*[*] | | PCR | Xpert™ | | | | |
| | NHP | Species | Intracellular | Extracellular | Intracellular | Extracellular | INS1e2 | HSP | IS1081/ IS16110 | Lung | Lymph node | Liver | Spleen |
| Old World Monkeys | 1 | *Macaca mulatta* | + [a] | ++ [a] | +++ [a] | + [a] | P [a] | N | P [a] | P | P | P | P |
| | 2 | *Macaca mulatta* | + [e] | + [e] | +++ [e] | ++ [e] | P [a] | N | P [a] | P | P | NP | P |
| | 3 | *Macaca mulatta* | + [a] | + [a] | ++ [a] | - | P [a] | N | P [a] | P | NP | NP | N |
| | 4 | *Macaca mulata* | ++ [a] | + [a] | ++ [a] | +++ [a] | P [a] | P [a] | P [a] | P | P | N | P |
| | 5 | *Macaca mulatta* | - | - | + [a] | + [a] | P [a] | N | P [a] | N | NP | N | P |
| | 6 | *Macaca mulatta* | - | - | + [a] | - | P [a] | P [a] | P [a] | P | P | N | N |
| | 7 | *Macaca mulatta* | - | - | ++ [a] | ++ [a] | N | N | P [a] | NP | NP | NP | NP |
| | 8 | *Macaca fascicularis* | - | - | + [b] | - | P [b] | N | NP | NP | NP | NP | NP |
| | 9 | *Macaca fascicularis* | + | - | +++ [e] | + [e] | NP | NP | NP | NP | NP | NP | NP |
| New World Monkeys | 10 | *Callithrix* spp. | + [a] | + [a] | +++ [a] | + [a] | P [a] | N | P [a] | NP | NP | NP | NP |
| | 11 | *Callithrix* spp. | + [a] | + [a] | + [a] | - | NP | NP | P [a] | NP | NP | NP | NP |
| | 12 | *Callithrix* spp. | + [a] | + [a] | +++ [a] | + [a] | P [c] | N | P [a] | NP | NP | NP | NP |
| | 13 | *Saimiri ustus* | - | - | + [a] | - | NP | NP | P [a] | NP | NP | NP | NP |
| | 14 | *Saimiri ustus* | + [d] | - | ++ [d] | - | NP | NP | P [d] | NP | NP | NP | NP |
| | 15 | *Chiropotes utahickae* | + [a] | ++ [a] | +++ [a] | ++ [a] | NP | NP | NP | NP | NP | NP | NP |

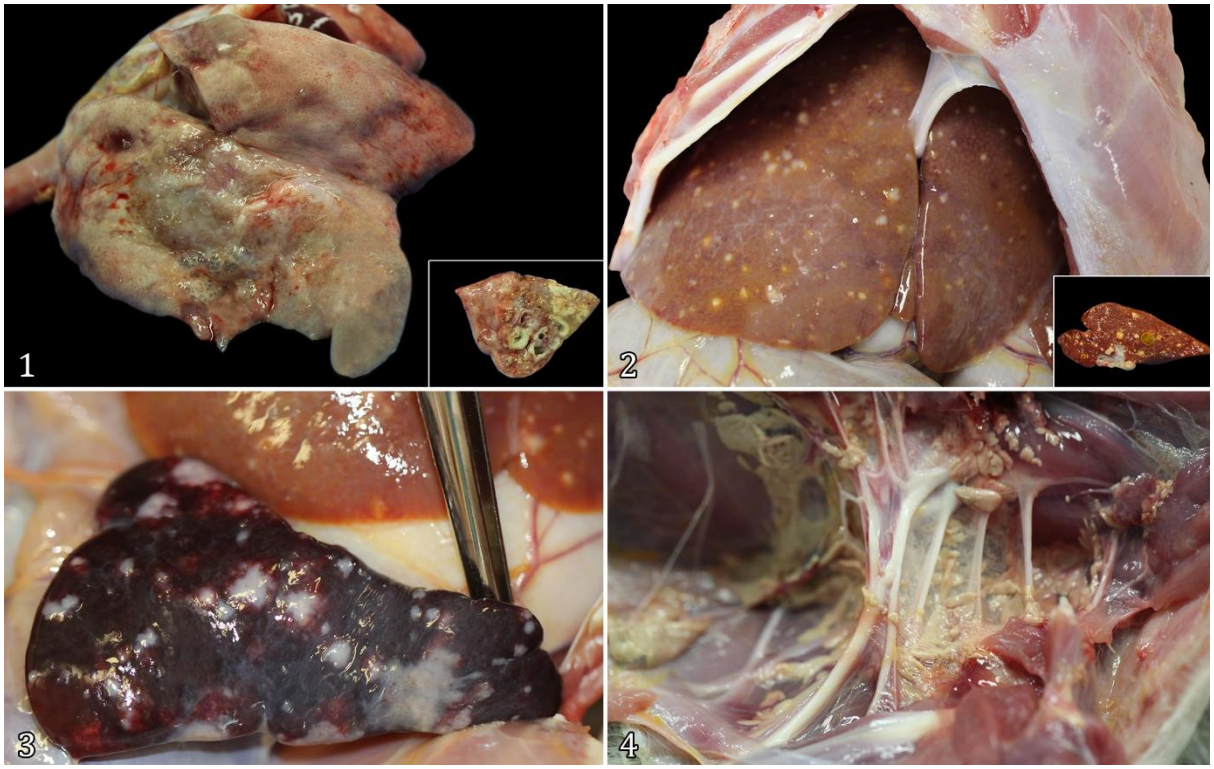
(-) Absent; (+) Mild; (++) Moderate; (+++) Severe; (P) Positive; (N) Negative; (NP) Not performed; [*] Polyclonal antibody anti-*Mycobacterium tuberculosis* (Bioscience Inc®, San Diego, CA, USA); [#] *Mycobacterium tuberculosis* Complex; [a] Lung; [b] Heart; [c] Pleura and striated muscle; [d] Liver; [e] Lymph node.

**Table 5 – Stage of tuberculosis in New and Old World Monkeys**

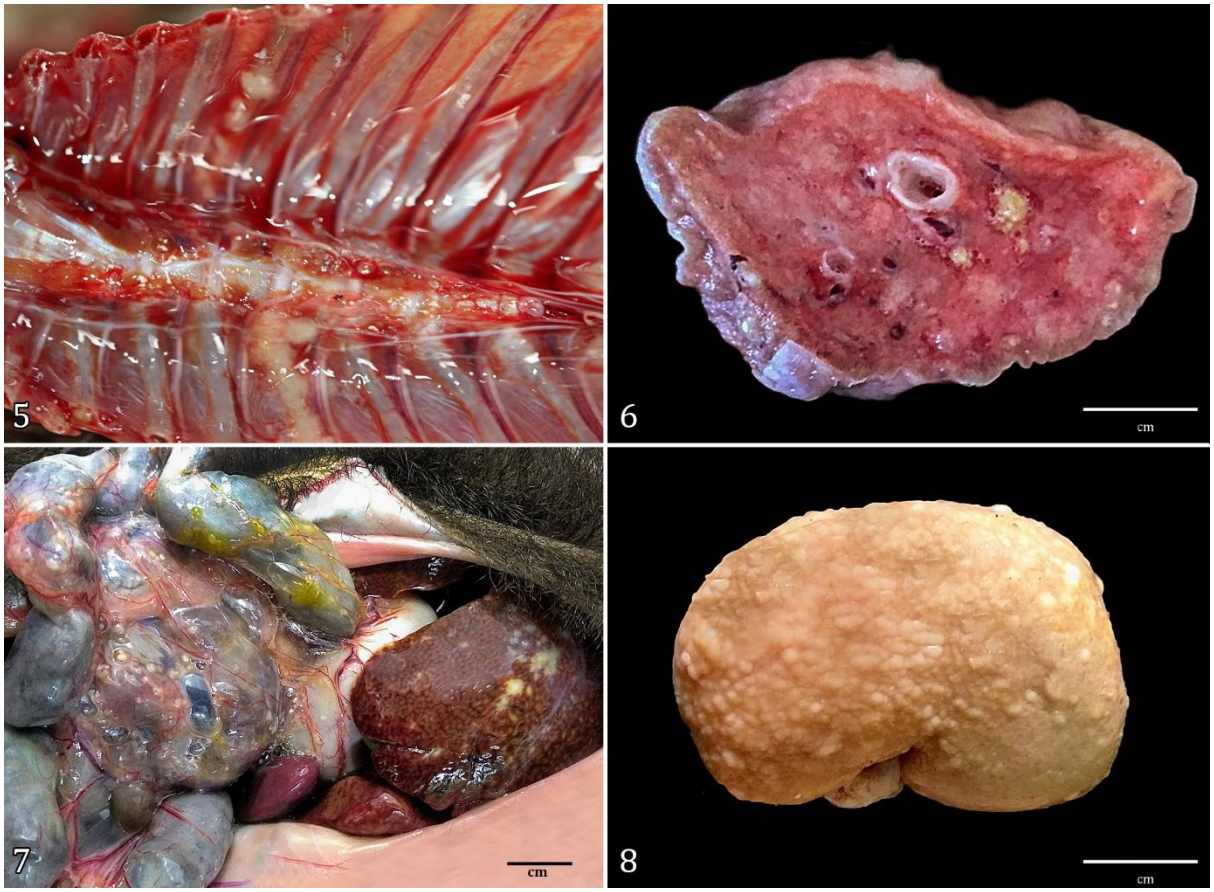
| | NHP | Species | Stage of tuberculosis | Respiratory Signs | Typical Lung granuloma | | Extrapulmonary involvement |
|---|---|---|---|---|---|---|---|
| | | | | | Gross | Microscopic | |
| Old World Monkeys | 1 | *Macaca mulatta* | Active-chronic | + | + | + | Multisystemic |
| | 2 | *Macaca mulatta* | Extrapulmonary | + | - | - | Multisystemic |
| | 3 | *Macaca mulatta* | Active-chronic | - | + | + | Multisystemic |
| | 4 | *Macaca mulata* | Active-chronic | + | + | + | Multisystemic |
| | 5 | *Macaca mulatta* | Active-chronic | + | + | + | Multisystemic |
| | 6 | *Macaca mulatta* | Active-chronic | + | + | + | Multisystemic |
| | 7 | *Macaca mulatta* | Active-chronic | - | + | + | Multisystemic |
| | 8 | *Macaca fascicularis* | Extrapulmonary | - | - | - | Heart |
| | 9 | *Macaca fascicularis* | Extrapulmonary | - | - | - | Lymph node |
| New World Monkeys | 10 | *Callithrix* spp. | Early activation | - | - | - | - |
| | 11 | *Callithrix* spp. | Early activation | - | - | - | - |
| | 12 | *Callithrix spp.* | Extrapulmonary | - | - | - | Multisystemic |
| | 13 | *Saimiri ustus* | Early activation | + | - | - | - |
| | 14 | *Saimiri ustus* | Extrapulmonary | - | - | - | Liver |
| | 15 | *Chiropotes utahickae* | Latent-reactivation | - | + | + | Multisystemic |

(-) Absent; (+) Present.

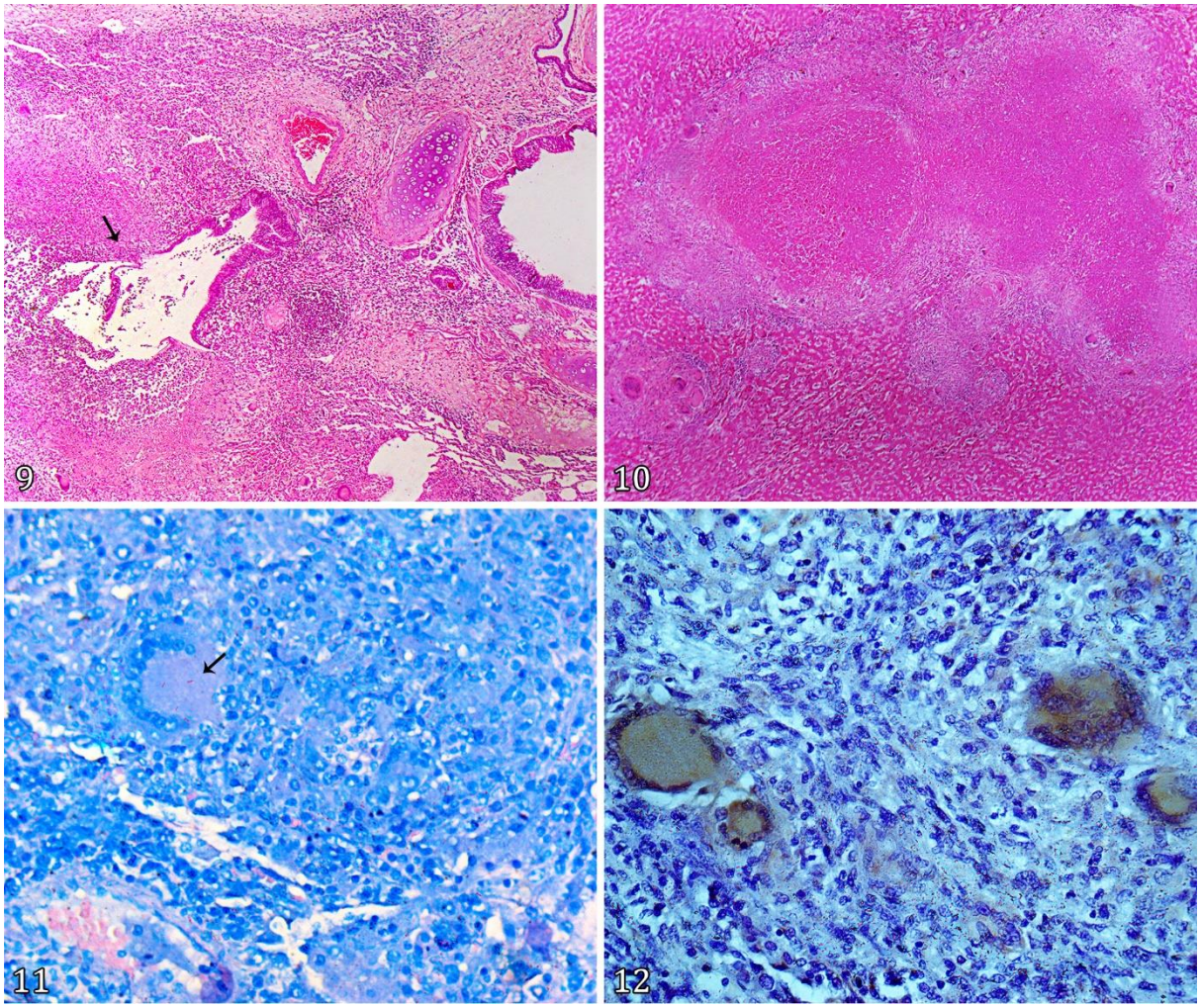
# Figure caption



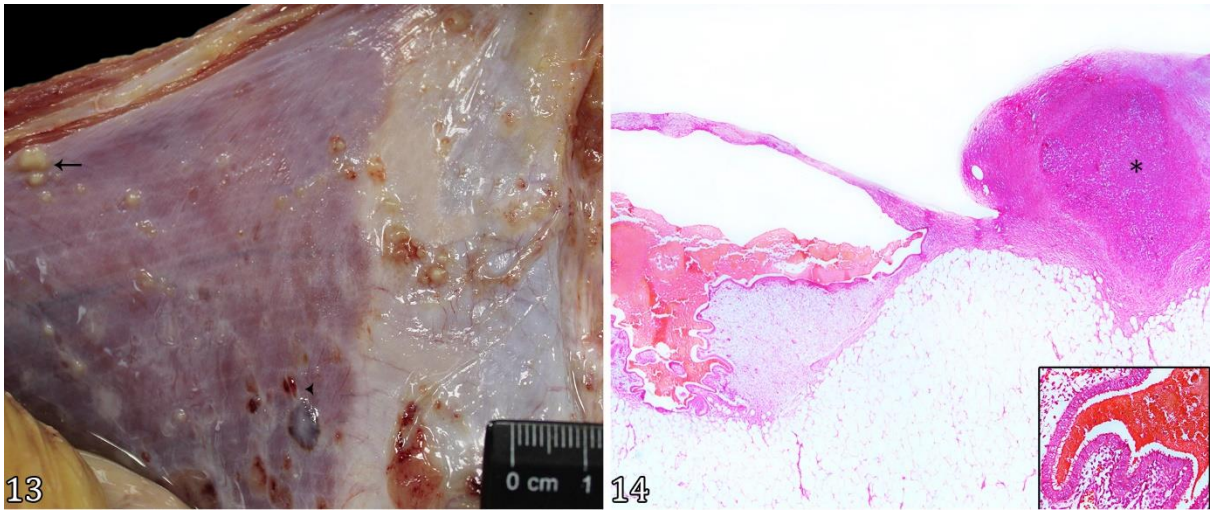

**Figures 1-4.** Gross findings of rhesus monkey (*Macaca mulatta*) with chronic-active tuberculosis stage. **Figure 1.** Multifocal to coalescent granulomatous pneumonia. with well-demarcated structures, sometimes elevated, firm yellow-white or grayish nodules of different sizes. The lungs are diffusely firm, enlarged and reddish with multiple consolidations areas. Inset: multiple cavitation areas of different sizes on the airways **Figure 2.** Multifocal granulomatous hepatitis. Inset: hepatic cut surface with multifocal granulomas of variable size on parenchyma **Figure 3.** Multifofcal granulomatous splenitis. **Figure 4.** Multifocal granulomatous neuritis. There is a great number of granulomas adhered on the nerves sheets.



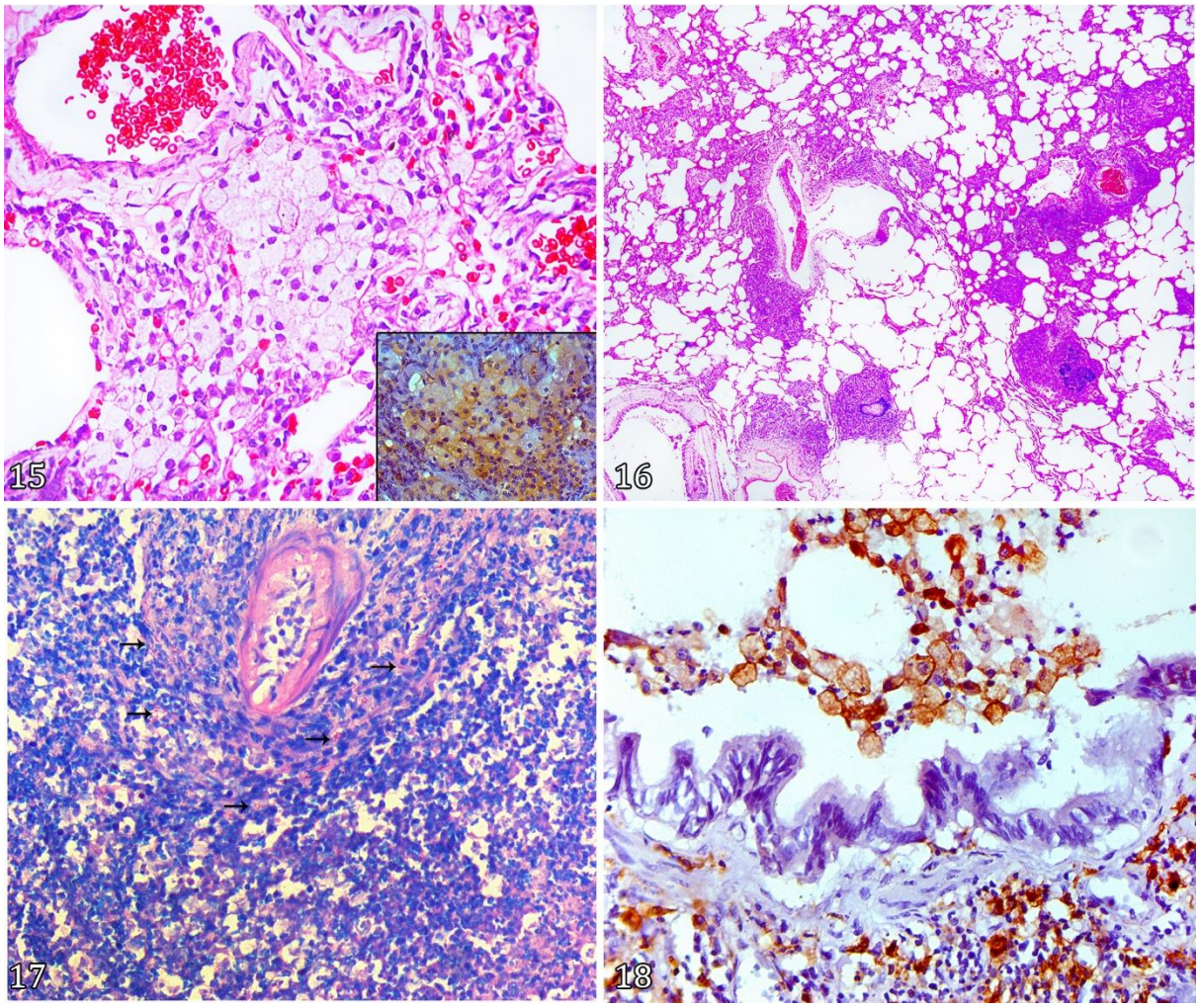

**Figures 5-8.** Gross findings of New World Monkeys with different tuberculosis stages. **Figure 5.** Under the parietal pleura and skeletal muscle, multifocal areas of granulomas formation in a *Callithrix* sp. with extrapulmonary tuberculosis. **Figure 6.** Lung cut surface of an Uta Hick's Bearded Saki with latent-reactivation tuberculosis stage. Note the multifocal areas with well demarked yellow-wish areas n the airways. **Figure 7.** Systemic granulomatous spread of an Uta Hick's Bearded Saki with latent-reactivation tuberculosis stage. Note granulomatous hepatitis and mesenteric granulomatous lymphadenitis. **Figure 8.** Multifocal granulomatous nephritis of an Uta Hick's Bearded Saki with latent-reactivation tuberculosis stage.




**Figures 9-12.** Microscopic findings of Rhesus Monkey (*Macaca mulatta*) with chronic-active tuberculosis stage. **Figure 9.** Granulomatous severe pneumonia. The airways are replaced by epithelioid macrophages, multinucleated giant cells (Langhans and Foreign body giant cells), a variable number of lymphocytes, neutrophils and rarely foam cells. There is a focal area of ulceration of bronchial epithelial cells and cavitation to the airways (arrow). There are multifocal areas of necrosis with a great number of pyknotic debris and hyperosinophilic amorphous material. (HE, Obj 2,5x). **Figure 10.** Granulomatous hepatitis with a central large caseous granuloma. (HE, Obj. 5x). **Figure 11.** Few acid-fast bacilli into the cytoplasm of giant cell multinucleated (arrow) and in the extracellular medium. (Ziehl-Neelsen, Obj. 40x). **Figure 12.** Intense intracytoplasmic imunolabeling anti-*Mycobacterium tuberculosis* in the giant cells multinucleated with evidence of intact and degenerated bacilli in the cytoplasm. Besides, note the great number of bacilli into the extracellular medium (IHC anti-Mycobacterium tuberculosis, Obj. 40x).




**Figures 13-14.** Pathological findings of Rhesus Monkey (*Macaca mulatta*) with conconmitant extrapulmonary tuberculosis and endometriosis. **Figure 13.** Gross finding of abdominal wall with granulomatous white nodules (arrow) and dark-red cystic masses (arrowhead). **Figure 14.** Concomitance of endometrial cystic proliferation and a caseous granuloma (asterisk) (HE, Obj. 2,5x). Inset: moderately cellular proliferation composed of columnar and ciliated endometrial cells with a moderate amount of eosinophilic cytoplasm and round nuclei. (HE, Obj. 40x).




**Figures 15-18.** Microscopic findings of New World Monkeys with different tuberculosis stages. **Figure 15.** Interstitial foamy pneumonia in a *Callithrix* sp. with early-activation tuberculosis. The septa are distended by a great number of foamy macrophages and lymphocytes. (HE, Obj 40x). Inset: Intense intracytoplasmic imunolabeling anti-*Mycobacterium tuberculosis* in foamy macrophages with evidence of intact and degenerated bacilli in the cytoplasm. (IHC anti-Mycobacterium tuberculosis, Obj. 40x). **Figure 16.** Multifocal solid-cellular and mineralized granulomatous with some areas of caseous necrosis in the lung of Uta Hick's Bearded Saki (*Chiropotes utahickae*) with latent-reactivation tuberculosis stage (HE, Obj. 2,5x). **Figure 17.** Acid-fast bacilli into the extracellular medium (arrow) of a caseous lung granuloma of an Uta Hick's Bearded Saki (*Chiropotes utahickae*) with latent-reactivation tuberculosis stage. (Ziehl-Neelsen, Obj. 40x). **Figure 18.** Intense intracytoplasmic imunolabeling anti-*Mycobacterium tuberculosis* in the intrabronchial foamy macrophages with evidence of intact and degenerated bacilli in the cytoplasm. Besides, note the great number of bacilli into the intra and extracellular medium at the submucosa of bronchi (IHC anti-Mycobacterium tuberculosis, Obj. 40x).

## 4. REFERÊNCIAS BIBLIOGRÁFICAS

ABER V. R.; NUNN, A. J. Short term chemotherapy of tuberculosis. Factors affecting relapse following short term chemotherapy. **Bulletin of the International Union against Tuberculosis** v. 53, n. 4, p. 276-280, 1978.

ACHARYA, B.; ACHARYA, A.; GAUTAM, S.; GHIMIRE, S. P.; MISHRA, G.; PARAJULI, N.; SAPKOTA, B. Advances in diagnosis of Tuberculosis: an update into molecular diagnosis of *Mycobacterium tuberculosis*. **Molecular Biology Reports**, v. 47, n. 5, p. 4065–4075, 2020.

ALLEN, A. M.; KINARD, R. F. Primary cutaneous inoculation tuberculosis in the *Macaca mulatta* monkey. **The American Journal Of Pathology,** v. 34, n. 2 , p. 337, 1958.

AREND, S. M.; SOOLINGEN, D. Performance of Xpert MTB/ RIF Ultra: a matter of dead or alive. **The Lancet Infectious Diseases**, v.18, n. 1, p. 8-10, 2018.

ARMSTRONG, J. A; HART, P. D. Phagosome-lysosome interactions in cultured macrophages infected with virulent tubercle bacilli: reversal of the usual nonfusion pattern and observations on bacterial survival. **The Journal of experimental medicine**, v. 142, n. 1, p. 1-16, 1975.

BARCLAY, W. R.; ANACKER, R. L.; BREHMER, W.; LEIF, W.; RIBI, E. Aerosol-induced tuberculosis in subhuman primates and the course of the disease after intravenous BCG vaccination. **Infection and Immunity**, v. 2, n. 5, p. 574-582, 1970.

BARCLAY, W. R.; BUSEY, W. M.; DALGARD, D. W.; GOOD, R. C.; JANICKI, B. W.; KASIK, J. E.; RIBI, E.; ULRICH, C. E.; WOLINSKY. E. 1973. Protection of monkeys against airborne tuberculosis by aerosol vaccination with bacillus Calmette-Guerin. **American Review of Respiratory Disease,** v. 107, n. 3, p. 351-358, 1973.

BARRY, C. E.; LEE, R. E.; MDLULI, K.; SAMPSON, A. E.; SCHROEDER, B. G.; SLAYDEN, R. A.; YUAN, Y. S.; Ying, Y. Mycolic acids: structure, biosynthesis and physiological functions. **Progress in Lipid Research** v. 37, n. 2-3, p. 143-179, 1998.

BARRY, C. E.; BOSHOFF, H. I.; DARTOIS, V.; DICK, T.; EHRT, S.; FLYNN, J.; SCHNAPPINGER, D.; WILKINSON, R. J.; YOUNG, D. 2009. The spectrum of latent tuberculosis: rethinking the biology and intervention strategies. **Nature Reviews Microbiology**, v. 7, n. 12, p. 845-855, 2009.

BASARABA, R. J. Experimental tuberculosis: the role of comparative pathology in the discovery of improved tuberculosis treatment strategies. **Tuberculosis**, v. 88, n. SUPPL. 1, 2008.

BEHAR, S. M.; DASCHER, C. C.; GRUSBY, M. J.; WANG, C. R.; BRENNER, M. B. Susceptibility of mice deficient in CD1D or TAP1 to infection with *Mycobacterium tuberculosis*. **The Journal of Experimental Medicine,** v. 189, n. 12 p. 1973-1980, 1999.

BONE, J. F.; SOAVE, O. A. Experimental tuberculosis in owl monkeys (*Aotus trivirgatus*). **Laboratory Animal Care,** v. 20, n. 5, p. 946– 948, 1970.

BRACK, M. **Agents transmissible from simians to man**. Springer Verlag, Berlin, Heidelberg, New York. 1987

BRAMMER, D. W.; O'ROURKE, C. M.; HEATH, L. A.; CHRISP, C. E.; PETER, G. K.; HOFING, G. L. *Mycobacterium kansasii* infection in squirrel monkeys (*Saimiri sciureus sciureus*). Journal of medical primatology 24, no. 4 (1995): 231-235.

BRASIL. Ministério da Saúde. Secretaria de Vigilância em Saúde. Departamento de Doenças de Condições Crônicas e Infecções Sexualmente Transmissíveis – DCCI. **Boletim Epidemiológico Especial, Número Especial, Tuberculose**, 2020.

BURKHOLDER, C. R.; HIRSH, D. C.; HICKMAN, R. L.; SOAVE, O. A. Influence of isoniazid therapy on the course of tuberculosis in a rhesus monkey. **Journal of the American Veterinary Medical Association**, v. 151, n. 7, p. 918-919, 1967.

BUSHMITZ, M.; LECU, A.; VERRECK, F.; PREUSSING, E.; RENSING, S.; MÄTZ-RENSING, K. Guidelines for the prevention and control of tuberculosis in nonhuman primates: Recommendations of the European Primate Veterinary Association Working Group on Tuberculosis. **Journal of Medical Primatology,** v. 38, n. 1, p. 59–69, 2009.

CADENA, A. M.; KLEIN, E. C.; WHITE, A. G.; TOMKO, J. A.; CHEDRICK, C. L.; REED, D. S.; VIA, L. E.; LIN, P. L.; FLYNN, J. L. Very low doses of *Mycobacterium tuberculosis* yield diverse host outcomes in common marmosets (*Callithrix jacchus*). **Comparative Medicine**, v. 66, n. 5, p. 412–419, 2016.

CADENA, A. M.; FORTUNE, S. M.; FLYNN, J. L. Heterogeneity in tuberculosis. **Nature Reviews Immunology**, v. 17, n. 11, p. 691–702, 2017.

CAPUANO, S. V.; CROIX, D. A.; PAWAR, S.; ZINOVIK, A. MYERS, A.; LIN, P. L.; BISSEL, S.; FUHRMAN, C.; KLEIN, E.; FLYNN, J. L. 2003. Experimental *Mycobacterium tuberculosis* infection of cynomolgus macaques closely resembles the various manifestations of human *M. tuberculosis* infection. **Infection and immunity**, v. 71, n. 10, p. 5831-5844, 2003.

CARDONA, P. J. Reactivation or reinfection in adult tuberculosis: Is that the question? **International Journal of Mycobacteriology**, v. 5, n. 4, p. 400–407, 2016.

CLARKE, G. L. The relationship of hypersensitivity to shedding of *Mycobacterium tuberculosis* in experimentally infected *Macaca mulatta*. **American Review of Respiratory Disease,** v. 98, n. 3, p. 416-423, 1968.

CLARKE, G. L.; SCHMIDT, J. P. Effect of prophylactic isoniazid on early developing experimental tuberculosis in *Macaca mulatta*. **American Review of Respiratory Disease,** v. 100, n. 2, p. 224-227, 1969.

CLEMENS, D. L.; HORWITZ, M. A. The *Mycobacterium tuberculosis* phagosome interacts with early endosomes and is accessible to exogenously administered transferrin." **The Journal of Experimental Medicine**, v. 184, n. 4, p. 1349-1355, 1996.

DE BACKER, A. I.; MORTELE, K. J.; DE KEULENAER, B. L.; PARIZEL, P. M. Tuberculosis: epidemiology, manifestations, and the value of medical imaging in diagnosis. **JBR BTR,** v. 89, n. 5, p. 243, 2006.

DHEDA, K.; BARRY, C. E.; MAARTENS, G. Tuberculosis. **Lancet** v. 387, p. 1211–1226, 2016.

DILLEHAY, D. L.; HUERKAMP, M. J. Tuberculosis in a tuberculin negativein rhesus monkey (*Macaca mulatta*) on chemoprophylaxis. **Journal. of Zoo and Wildlife Medicine**, v. 21, p. 480-484, 1990.

DODD, P. J.; YUEN, C. M.; JAYASOORIYA, S. M.; VAN DER ZALM, M. M.; SEDDON, J. A. Quantifying the global number of tuberculosis survivors: a modelling study. **The Lancet Infectious Diseases**, v. 3099, n. 20, p. 1–9, 2021.

DRAIN, P. K.; Bajema, K. L.; Dowdy, D.; Dheda, K.; Naidoo, K.; Schumacher, Samuel G Incipient and Subclinical Tuberculosis: a Clinical Review of Early Stages and Progression of Infection The Spectrum of Tuberculosis Infection to Disease. **Clinical Microbiology Reviews**, p. 1–24, 2018.

DUNN, J. J.; STARKE, J. R.; REVELL, P. A. Laboratory diagnosis of *Mycobacterium tuberculosis* infection and disease in children. **Journal of Clinical Microbiology**, v. 54, n. 6, p. 1434–1441, 2016.

EHLERS, L. P.; BIANCHI, M. V.; ARGENTA, F. F.; LOPES, B. C.; TAUNDE, P. A.; WAGNER, P. G. C.; DRIEMEIER, D.; PAVARINI, S. P.; MAYER, F. Q.; SIQUEIRA, F. M.; SONNE, L.. *Mycobacterium tuberculosis* var. tuberculosis infection in two captive black capuchins (*Sapajus nigritus*) in Southern Brazil. **Brazilian Journal of Microbiology**, v. 51, n. 4, p. 2169–2173, 2020.

ELLNER, J. J. Tuberculosis. **Goldman's Cecil Medicine**: Twenty Fourth Edition, v. 2, p. 1939–1948, 2012.

FENHALLS, G.;; WONG, A.; BEZUIDENHOUT, J.; VAN HELDEN, P.; BARDIN, P.; LUKEY, P. T. In situ production of gamma interferon, interleukin-4, and tumor necrosis factor alpha mRNA in human lung tuberculous granulomas. **Infection and immunity**, v. 68, n. 5, p. 2827-2836, 2000.

FLYNN, J. L.; CAPUANO, S. V.; CROIX, D.; PAWAR, S.; MYERS, A.; ZINOVIK, A.; KLEIN, E. Nonhuman primates: A model for tuberculosis research. **Tuberculosis**, v. 83, n. 1–3, p. 116–118, 2003.

FONG, I. W. Emerging and Difficult to Treat Nontuberculous Mycobacteria Infections. **Current Trends and Concerns in Infectious Diseases**, 289-317. 2020

FORBES B. A.; SAHM, D. F.; WEISSFELD, A. S. Tuberculosis. In:**Bailey and Scott's Diagnostic Microbiology**, 10th edn. Mosby, St. Louis. 2000

FOREMAN, T. W.; MEHRA, S.; LACKNER, A. A.; KAUSHAL, D. Translational research in the nonhuman primate model of tuberculosis. **ILAR Journal**, v. 58, n. 2, p. 151–159, 2017.

FORRELLAD, M. A.; KLEPP, L. I.; GIOFFR´E, A.; SABIO, G. J.; MORBIDONI, H. R.; SANTANGELO, M. D. L. P.; CATALDI, A. A.; BIGI, F. Virulence factors of the *Mycobacterium tuberculosis* complex. **Virulence**, v. 4, n. 1, p. 3–66, 2013.

FREMMING, B. D.; BENSON, R. E.; YOUNG, R. J.; HARRIS, M. D. Antituberculous therapy in *Macaca mulatta* monkeys. **American Review of Tuberculosis and Pulmonary Diseases**, v. 76, n. 2, p. 225-231, 1957.

GARCIA, M. A.; YEE, J.; BOULEY, D. M.; MOORHEAD, R.; LERCHE, N. W. Diagnosis of tuberculosis in macaques, using whole-blood in vitro interferon-gamma (PRIMAGAM) testing. **Comparative medicine**, v. 54, n. 1, p. 86-92, 2004.

GHODBANE, R.; DRANCOURT, M. Non-human sources of *Mycobacterium tuberculosis*. **Tuberculosis**, v. 93, n. 6, p. 589–595, 2013.

GOOD R. C. Biology of the mycobacterioses. Simian tuberculosis: immunologic aspects. **Annals of the New York Academy of Sciences,** v. 154, n. 1, p. 200-213, 1968.

GOOD, R. C. Diseases in nonhuman primates. In **The mycobacteria: A sourcebook** (ed. Kubica GP, Wayne LG), p. 903–921. Dekker, New York, 1984.

HABERLE, A. J. Tuberculosis in an orangutan. **The Journal of Zoo Animal Medicine,** v. 1, no. 2, p. 10-15, 1970.

HALDAR S.; BOSE, M.; CHAKRABARTI, P.; DAGINAWALA, H. F.; HARINATH, B. C.; KASHYAP R. S. Improved laboratory diagnosis of tuberculosis - the Indian experience. **Tuberculosis**, v. 91, n. 5, p. 414-426, 2011.

HENRICH, M.; MOSER, I.; WEISS, A.; REINACHER, M. Multiple Granulomas in Three Squirrel Monkeys (*Saimiri sciureus*) Caused by *Mycobacterium microti*. **Journal of Comparative Pathology**, v. 137, n. 4, p. 245–248, 2007.

HERNANDEZ-PANDO, R.; JEYANATHAN, M.; MENGISTU, G.; AGUILAR, D.; OROZCO, H.; HARBOE, M.; BJUNE, G. Persistence of DNA from *Mycobacterium tuberculosis* in superficially normal lung tissue during latent infection. **The Lancet,** v. 356, n. 9248, p. 2133-2138, 2000.

HINES, M. E.; KREEGER, J. M.; HERRON, A. J. Mycobacterial infections of animals: pathology and pathogenesis. **Laboratory Animal Science**, v. 45, n.4, p. 334– 351, 1995.

INDZHILA, L. V.; YAKOVLEVA, L. A.; SIMOVONJAN, V. G.; DSHIKIDZE, E. K.; KOVALJOVA, I.; POPOVA, V. N. The character and results of comparative experimental therapy of tuberculosis in *Macaca arctoides* monkeys. **Immunity**, v. 19, p. 13-25, 1977.

INGEN, J. V.; RAHIM, Z.; MULDER, A.; BOEREE, M. J.; SIMEONE, R.; BROSCH, R.; SOOLINGEN, D. Characterization of *Mycobacterium orygis* as *M. tuberculosis* complex subspecies. **Emerging Infectious Diseases**, v. 18, n. 4, p. 653–655, 2012.

JANICKI, B.W.; GOOD R.C.; MINDEN, P.; AFFRONTI, L. F.; HYMES, W. F. Immune responses in rhesus monkeys after bacillus Calmette-Guerin vaccination and aerosol challenge with *Mycobacterium tuberculosis*. **American Review of Respiratory Disease**, v. 107, n. 3, p. 359-366, 1973.

KANABALAN, R. D.; LEE, L. J.; LEE, T. Y.; CHONG, P. P.; HASSAN, L.; ISMAIL, R.; CHIN, V. K. Human tuberculosis and *Mycobacterium tuberculosis* complex: A review on genetic diversity, pathogenesis and omics approaches in host biomarkers discovery. Microbiological Research, v. 246, e.126674, 2021.

KAPLAN, G.; POST, F. A.; MOREIRA, A. L.; WAINWRIGHT, H.; KREISWIRTH, B. N.; TANVERDI, M.; BEKKER, L. G. *Mycobacterium tuberculosis* growth at the cavity surface: a microenvironment with failed immunity. **Infection and immunity**, v. 71, n. 12, p. 7099-7108, 2003.

KARIMI, S. SHAMAEI, M.; POURABDOLLAH, M.; SADR, M.; KARBASI, M.; KIANI, A. BAHADORI, M. Immunohistochemical findings of the granulomatous reaction associated with tuberculosis**. International Journal of Mycobacteriology**, v. 5, p. 234–235, 2016.

KIM, M. J.; WAINWRIGHT, H. C.; LOCKETZ, M.; BEKKER, L. G.; WALTHER, G. B.; DITTRICH, C.; RUSSELL, D. G. Caseation of human tuberculosis granulomas correlates with elevated host lipid metabolism. **EMBO Molecular Medicine**, v. 2, no. 7 p. 258-274, 2010.

KOCK, R.; MICHEL, A. L.; YEBOAH-MANU, D.; AZHAR, E. I.; TORRELLES, J. B.; CADMUS, SIMEON, I.; BRUNTON, L.; CHAKAYA, J. M.; MARAIS, B.; MBOERA, L.; RAHIM, Z.; HAIDER, N. Z. Zoonotic Tuberculosis – The Changing Landscape. **International Journal of Infectious Diseases**, e342293. 2021.

KOLK A. H. J.; SCHUITEMA, R. J.; KUIJPER, S.; VAN LEEUWEN, J.; HERMANS, W. M.; VAN EMBDEN, J. D. A. Detection of *Mycobacterium tuberculosis* in clinical samples by using polymerase chain reaction and a nonradioactive detection system. **Journal of Clinical Microbiology** v. 30, n. 10, p. 2567-2575, 1992.

LAWN S. D.; NICOL, M. P. Xpert® MTB/RIF assay: development, evaluation and implementation of a new rapid molecular diagnostic for tuberculosis and rifampicin resistance. **Future Microbiology**, v. 6, n. 9, p. 1067–1082, 2011.

LENAERTS, A.; BARRY, C. E.; DARTOIS, V. Heterogeneity in tuberculosis pathology, microenvironments and therapeutic responses. **Immunological Reviews**, v. 264, n. 1, p. 288–307, 2015.

LIN, P. L.; PAWAR, S.; MYERS, A.; PEGU, A.; FUHRMAN, C.; REINHART, T. A.; CAPUANO, S. V.; KLEIN, E.; FLYNN, J. L. Early events in *Mycobacterium tuberculosis* infection in cynomolgus macaques. **Infection and immunity,** v. 74, p. 3790–3803, 2006.

LIN, P. L.; YEE, J., KLEIN, E; LERCHE, N. W. Immunological concepts in tuberculosis diagnostics for nonhuman primates: A review. **Journal of Medical Primatology**, v. 37, n. SUPPL.1, p. 44–51, 2008.

LIN, P. L.; RODGERS, M.; SMITH, L.; BIGBEE, M.; MYERS, A.; BIGBEE, C.; CHIOSEA, I.; CAPUANO, S. V.; FUHRMAN, C.; KLEIN, E.; FLYNN, J. L. Quantitative comparison of active and latent tuberculosis in the cynomolgus macaque model. **Infection and immunity**, v. 77, p. 4631–4642, 2009

MARTINEZ F.O.; HELMING, L.; GORDON, S. Alternative activation of macrophages: an immunologic functional perspective. **Annual Review of Immunology**, v. 27, p. 451–483, 2009.

MÄTZ-RENSING, K.; Hartman, T.; Wendel, G. M.; Frick, J. S; Homolka, S.; Richter, E.; Munk, M. H.; Kaup, F-J.. Outbreak of Tuberculosis in a Colony of Rhesus Monkeys (*Macaca mulatta*) after Possible Indirect Contact with a Human TB Patient. **Journal of Comparative Pathology**, v. 153, n. 2–3, p. 81–91, 2015.

MÄTZ-RENSING, K.; LOWENSTINE, L. J. New World and Old World monkeys. In: Terio KA, McAloose D, St. Leger J (eds) **Pathology of Wildlife and Zoo Animals**. Academic Press, San Diego, p. 343– 374, 2018.

MANABE, Y. C.; DANNENBERG, A.M. JR.; TYAGI, S.K.; HATEM, C. L.; YODER, M.; WOOLWINE, S. C. Different strains of *Mycobacterium tuberculosis* cause various spectrums of disease in the rabbit model of tuberculosis. **Infection and immunity**, v. 71, n. 10, p. 6004-6011, 2003.

Manabe YC, Kesavan AK, Lopez-Molina J.; Hatem, C. L.; Brooks, M.; Fujiwara, R. The aerosol rabbit model of TB latency, reactivation and immune reconstitution inflammatory syndrome. **Tuberculosis**, v. 88, n. 3, p. 187-196, 2008.

MANSFIELD, K. G.; FOX, J. G. Bacterial diseases. In: **The Common Marmoset in Captivity and Biomedical Research**. Academic Press, p. 265-287, 2019.

MAZUREK, G. H.; VILLARINO, M. E. Guidelines for using the QuantiFERON-TB test for diagnosing latent *Mycobacterium tuberculosis* infection. **Morbidity and mortality weekly report 52**, n. RR02, 2003.

MCCUNE, R. M.; FELDMANN, F. M.; MCDERMOTT, W. Microbial persistence, II: characteristics of the sterile state of tubercle bacilli. **The Journal of experimental medicine**, v. 123, n. 3, p. 469-486, 1966.

MCCLURE, H. M. Bacterial diseases of nonhuman primates. In R. J. Montali, G. Migaki (Eds.), **The Comparative Pathology of Zoo Animals**. Washington, D.C: Smithsonian Institution Press, p. 197-217,.1980.

MCLAUGHLIN, R. M.; MARRS, G. E. Tuberculin testing in nonhuman primates: OT vs. PPD. In R. M. Montali (Ed.), **Mycobacterial infections of zoo animals**. Washington, D.C: Smithsonian Institution Press, p. 123-128, 1978.

MCMURRAY DN. Guinea Pig Model of Tuberculosis. Washington, DC: **American Society for Microbiology**, 1994.

MCMURRAY DN. A nonhuman primate model for preclinical testing of new tuberculosis vaccines. **Clinical Infectious Disease**, v.30, n. Suppl. 3, p. S210e2, 2000.

MCMURRAY DN. Disease model: pulmonary tuberculosis. **Trends in Molecular Medice**., v, 7., n.9, p. 135–137, 2001.

MEHRA, S.; ALVAREZ, X.; DIDIER, P. J.; DOYLE, L. A.; BLANCHARD, J. L.; LACKNER, A. A.; KAUSHAL, D. Granuloma correlates of protection against tuberculosis and mechanisms of immune modulation by Mycobacterium tuberculosis. **Journal of Infectious Disease**, v. 207, n. 7, p. 1115–1127, 2013.

MONTALI, R. J.; MIKOTA, S. K.; CHENG, L. I. 2001. *Mycobacterium tuberculosis* in zoo and wildlife species. **Scientific and Technical Review, International Office of Epizootics**, v. 20, n.8, p. 291 –303, 2001.

MORELAND, A. F. Tuberculosis in New World primates. **Laboratory Animal Care**, v. 20, n. 2, p. 262–264, 1970.

MUSTAFA, T. WIKER, H. G.; MFINANGA, S. G.M.; MØRKVE, O.; SVILAND, L. Immunohistochemistry using a *Mycobacterium tuberculosis* complex specific antibody for improved diagnosis of tuberculous lymphadenitis. **Modern Pathology**, v. 19, n. 12, p. 1606–1614, 2006.

NESHANI, A.; KAKHKI, R. K.; SANKIAN, M.; ZARE, H.; CHICHAKLU, A. H.; SAYYADI, M.; GHAZVINI, K. Modified genome comparison method: A new approach for identification of specific targets in molecular diagnostic tests using *Mycobacterium tuberculosis* complex as an example. **BMC Infectious Diseases**, v. 18, n. 1, p. 1–9, 2018.

NEYROLLES, O.; HERNANDEZ-PANDO, R.; PIETRI-ROUXEL, F.; FORNÈS, P.; TAILLEUX, L.; PAYÁN, J. A. B. Is adipose tissue a place for *Mycobacterium tuberculosis* persistence? **PLoS One**, v.1, n. 43, 2006.

OBALDÍA, N.; NUÑEZ, M.; MONTILLA, S.; OTERO, W.; MARIN, J. C. Tuberculosis (TB) outbreak in a closed *Aotus* monkey breeding colony: Epidemiology, diagnosis and TB screening using antibody and interferon-gamma release testing. **Comparative Immunology, Microbiology and Infectious Diseases**, v. 58, n. 5, p. 1–10, 2018.

PAI, M.; RILEY, L. W.; COLFORD, J. M. Interferon-gamma assays in the immunodiagnosis of tuberculosis: a systematic review. **The Lancet Infectious Diseases,** v. 4, n. 12, p. 761-776, 2004.

PARK, M.K.; MYERS, R. A.; MARZELLA, L. Oxygen tensions and infections: modulation of microbial growth, activity of antimicrobial agents, and immunologic responses. **Clinical Infectious Disease**, v. 14, n. 3, p. 720–740, 1992.

PARSONS, S. D.; GOUS, T. A.; WARREN, R. M.; DE VILLIERS, C.; SEIER, J. V.; VAN HELDEN, P. D. Detection of *Mycobacterium tuberculosis* infection in chacma baboons (*Papio ursinus*) using the QuantiFERONTB gold (in-tube) assay. **Journal of medical primatology**, v. 38, n. 6, p. 411-417, 2009.

PEYRON, P.; VAUBOURGEIX, J.; POQUET, Y.; LEVILLAIN, F.; BOTANCH, C.; BARDOU, F.; ALTARE, F. Foamy macrophages from tuberculous patients' granulomas constitute a nutrientrich reservoir for *M. tuberculosis* persistence. **PLoS Pathog**, v. 4, n. 11, e1000204, 2008.

PFYFFER, G. E. *Mycobacterium*: general characteristics, laboratory detection, and staining procedures**. Manual of Clinical Microbiology**, Eleventh Edition. American Society of Microbiology, p. 536–569, 2015

PIERCE, D. L.; DUKELOW, W. R. Misleading positive tuberculin reactions in a squirrel monkey colony. **Laboratory. Animal Science**., v. 38, n, 16, p. 729-730, 1988.

RAJ, A.; SINGH, N.; GUPTA, K. B.; CHAUDHARY, D.; YADAV, A.; CHAUDHARY, A.; YADAV, A.; MEHTA, P. K. Comparative evaluation of several gene targets for designing a multiplexPCR for an early diagnosis of Extrapulmonary tuberculosis. **Yonsei Medical Jornal**, v. 57, n. 1, p. 88-96, 2016.

REED, S. G.; COLER, R. N.; DALEMANS, W.; TAN, E. V.; CRUZ, E. C. D.; BASARABA, R. J.; LOBET, Y. 2009. Defined tuberculosis vaccine, Mtb72F/AS02A, evidence of protection in cynomolgus monkeys. **Proceedings of the National Academy of Sciences**, v. 106, n. 7, p. 2301-2306, 2009.

RENQUIST, D. M., & WHITNEY, R. A. Tuberculosis in nonhuman primates - an overview. In R. J. Montali (Ed.), **Mycobacterial Infections in Zoo Animals**. Washington, DC: Smithsonian Institution Press, p. 9-16, 1978.

REZNIK GK. Comparative anatomy, physiology, and function of the upper respiratory tract. **Environmental Health Perspective**, v. 85, n.2, p. 171–176, 1990.

RIORDAN, J. T. Rectal tuberculosis in monkeys from the use of contaminated thermometers. **Journal of Infectious Diseases**, v. 73, n. 2, p. 93–94, 1943.

RIDLEY, D. S.; RIDLEY, M. J. Rationale for the histological spectrum of tuberculosis. A basis for classification. **Pathology,** v. 19, n. 9, p. 186–192, 1987

RILEY R. L. Aerial dissemination of pulmonary tuberculosis. **American Review of Tuberculosis**, v. 76, p. 931–941, 1957.

ROCHA, V. C. M.; IKUTA, C. Y.;GOMES, M. S.; QUAGLIA, F.; MATUSHIMA, E. R.; NETO, J. S. F. Isolation of mycobacterium tuberculosis from captive ateles paniscus. **Vector-Borne and Zoonotic Diseases**, v. 11, n. 5, p. 593–594, 2011.

RODRIGUEZ, J. G.; MEJIA, G. A.; DEL PORTILLO, P.; PATARROYO, M. E.; MURILLO, L. A. Species-specific identification of *Mycobacterium bovis* by PCR. **Microbiology**, v. 141 n. 9, p. 131-138, 1995.

ROJAS-ESPINOSA O.; DANNENBERG JR, A. M.; STERNBERGER, L. A.; TSUDA, T. The role of cathepsin D in the pathogenesis of tuberculosis. A histochemical study employing unlabeled antibodies and the peroxidase-antiperoxidase complex. **The American Journal of Pathology**, v. 74, n. 1, p. 1, 1974.

SAKAMOTO, K. The Pathology of *Mycobacterium tuberculosis* Infection. **Veterinary Pathology**, v. 49, n. 3, p. 423–439, 2012.

SCANGA, C. A.; FLYNN, J. L. Modeling tuberculosis in nonhuman primates. **Cold Spring Harbor Perspectives in Medicine**, v. 4, n. 12, p. 1–16, 2014.

SCHMIDT, L. H. Some observations on the utility of simian pulmonary tuberculosis in defining the therapeutic potentialities of isoniazid. **American Review of Tuberculosis**, v; 74, n.23, p. 138–159, 1956

SCHMIDT, L. H. Studies on the antituberculous activity of ethambutol in monkeys. **Annals of the New York Academy of Sciences,** v. 135, n. 2, p. 747-758, 1966.

SCHWANDER, S.; DHEDA, K. Human lung immunity against *Mycobacterium tuberculosis*: insights into pathogenesis and protection. **American Journal of Respiratory Critical Care Medicine**, v. 183, p. 696 –707, 2011.

SHI, S. R.; DATAR, R.; LIU, C.; WU, L.; ZHANG, Z.; COTE, R. J.; TAYLOR, C. R. DNA extraction from archival formalin-fixed, paraffin-embedded tissues: heat-induced retrieval in alkaline solution. **Histochemistry and Cell Biology,** v. 122, n.3, p. 211–218, 2004.

SHIPLEY, S. T.; COKSAYGAN, T.; JOHNSON, D. K.; MCLEOD, C. G.; DETOLLA, L. J. Diagnosis and prevention of dissemination of tuberculosis in a recently imported rhesus macaque (*Macaca mulatta*). **Journal of Medical Primatology**, v. 37, Suppl. 1, 20e4, 2008.

SIMMONS, J. H., GIBSON, S. Bacterial and mycotic diseases of nonhuman primates. In **Nonhuman primates in biomedical research**. Elsevier Inc, p. 105-172, 2012.

SMITH, N.H.; KREMER, K.; INWALD, J.; DALE, J.; DRISCOLL, J.R.; GORDON, S.V.; VAN SOOLINGEN, D.; HEWINSON, R.G.; SMITH, J.M. Ecotypes of the *Mycobacterium tuberculosis* complex. **Journal of Theorical Biology,** v. 239, n. 2, 2006220- 5, 2008.

SOHN, H.; AERO, A. D.; MENZIES, D.; BEHR, M.; SCHWARTZMAN, K.; ALVAREZ, G. G.; DENKINGER, C. M. Xpert MTB/RIF testing in a low tuberculosis incidence, high-resource setting: limitations in accuracy and clinical impact. **Clinical Infectious Disease**, v. 58, n. 7, p. 970–976, 2014.

TELENTI, A.; MARCHESI, F.; BALZ, M.; BALLY, F.; BOTTGER, E. C.; BODMER, T. Rapid identification of mycobacteria to the species level by polymerase chain reaction and restriction enzyme analysis. **Journal of Clinical Microbiology**, v. 31, n.2, p. 175–178, 1993.

TRIBE, G. W.; WELBURN, A. E. Value of combining the erythrocyte sedimentation rate test with tuberculin testing in the control of tuberculosis in baboons. **Laboratory Animals**, v. 10, n 19, p. 39-46, 1976.

ULRICHS, T.; KAUFMANN, S. H. New insights into the function of granulomas in human tuberculosis. **Journal of Pathology**, v. 208, n.4, p. 261–269, 2006

VIA, L. E.; LIN, P. L.; RAY, S. M.; CARRILLO, J.; ALLEN, S. S.; EUM, S. Y.; BARRY, C. E. Tuberculous granulomas are hypoxic in guinea pigs, rabbits, and nonhuman primates. **Infection and Immunity**, v.76, n.6, p. 2333–2340, 2008.

VIA, L. E.; WEINER, D. M.; SCHIMEL, D.; LIN, P. L.; DAYAO, E.; TANKERSLEY, S. L.; CAI, Y.; COLEMAN, M. T.; TOMKO, J.; PARIPATI, P.; ORANDLE, M.; KASTENMAYER, R. J.; TARTAKOVSKY, M.; ROSENTHAL, A.; PORTEVIN, D.; EUM, S. Y.; LAHOUAR, S.; GAGNEUX, S.; YOUNG, D. B.; FLYNN, J. L.; BARRY, C. E. Differential virulence and disease progression following mycobacterium tuberculosis complex infection of the common marmoset (*Callithrix jacchus*). **Infection and Immunity**, v. 81, n. 8, p. 2909–2919, 2013.

WALSH, G. P.; TAN, E. V; CRUZ, E. C. D.; ABALOS, R. M.; VILLAHERMOSA, L. G.; YOUNG, L. J.; HORWITZ, M. A. The Philippine cynomolgus monkey (*Macaca fasicularis*) provides a new nonhuman primate model of tuberculosis that resembles human disease. **Nature Medicine**, v. 2, n. 4, p. 430-436, 1996.

WARD, G. S.; ELWELL, M. R.;; TINGPALAPONG, M.; POMSDHIT, J. Use of streptomycin and isoniazid during a tuberculosis epizootic in a rhesus and cynomolgus breeding colony. **Laboratory. Animal. Science**, v. 35, n. 13, p. 395-399, 1985.

WARIT, S.; BILLAMAS, P.; MAKHAO, N.; JAITRONG, S.; JUTHAYOTHIN, T.; YINDEEYOUNGYEON, W.; DOKLADDA, K.; SMITTIPAT, N.; KEMTHONG, T.; MEESAWAT, S.; KONGSOMBAT, N.; KRAITAT, C.; PRAMMANANAN, T.; PALAGA, T.; CHAIPRASERT, A.; MALAIVIJITNOND, S. Detection of tuberculosis in cynomolgus macaques (*Macaca fascicularis*) using a supplementary Monkey Interferon Gamma Releasing Assay (mIGRA). **Scientific Reports**, v. 10, n. 1, p. 1–11, 2020.

WEISS, C.; TABACHNICK, J.; COHEN, H. P. Mechanism of softening of tubercles. III. Hydrolysis of protein and nucleic acid during anaerobic autolysis of normal and tuberculous lung tissue in vitro. **AMA Archives of Pathology**, v.57, n. 3, p. 179–193, 1954.

WHO. **Treatment of tuberculosis guidelines**, 4th ed. World Health Organization, Geneva, Switzerland. 2010.

WHO. Consensus meeting report: development of a target product profile (TPP) and a framework for evaluation for a test for predicting progression from tuberculosis infection to active disease. Geneva, Switzerland, 2017.

WHO. **Latent tuberculosis infection: updated and consolidated guidelines for programmatic management**. World Health Organization, Geneva, Switzerland, 2018.

WHO, WORLD HEALTH ORGANIZATION. **Global Tuberculosis Report**. Executive Summary, 2019

WHO. **Tuberculosis: Key facts**. Geneva, Switzerland; 2020a.

WHO. **Global Tuberculosis report**. Geneva, Switzerland; 2020b

WOLF, R. H.; GIBSON, S. V.; WATSON, E. A.; BASKIN, G. B. Multidrug chemotherapy of tuberculosis in rhesus monkeys. **Laboratory Animal Science**, v. 38, n. 9, p. 25-33, 1988.