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Zoonoses Tuberculosis Risk from Non-Human-Primate Trade and Movement: Preventive Measure Perspective

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Abstract: *The trade in non-human primates (NHPs) is economically profitable in both local and global markets. However, it also poses a significant threat of disease transmission between NHPs and humans, particularly zoonotic tuberculosis, which is frequently reported in NHPs. Due to the high similarity between NHPs and humans, they share many pathogens. As a potential hub for NHP trade, Indonesia needs to enhance its knowledge and preventive measures to manage tuberculosis transmission related to NHPs trade and movement. Legislation at both international and national levels has been established to address the prevention of tuberculosis infection from NHPs. In addition to legislative measures, effective prevention also relies on implementing screening and detection protocols, as well as maintaining good biosecurity practices during handling NHPs.*

Keyword: *Non-human-primate, tuberculosis, zoonoses, Indonesia.*

INTRODUCTION

Indonesia, a country renowned for its rich biodiversity, is home for 59 primates species and possesses the second-largest primate population in the world, following Brazil (Ruskhanidar *et al.*, 2017). Indonesia primates are unique due to their high biodiversity, ranging from prosimian (such as slow lorises and tarsiers) to various Old World monkey species (including macaques, langur, and proboscis monkeys), as well as lesser apes (like siamangs and gibbons) and great apes (such orang utan) (Ross *et al.*, 2014). These primates are distributed across Indonesia numerous islands. The country's biodiversity is classified into two major hotspots: Wallacea and Sundaland, which contribute to the high levels of endemism among its primate species) (Ruskhanidar *et al.*, 2017).

Non-human primates (NHPs) share a close genetic relationship with humans, with a genetic homology ranging from 75% to 98.5%. This similarity extends to tissue structures, immune systems, and metabolic processes, which means NHPs can host many of the same pathogens as humans. Consequently, NHPs are often used as model animals in the biomedical

industry and pharmaceutical research. Since the early 1970s, India has legally exported approximately 20,000 to 50,000 NHPs annually to meet the demand for biomedical studies. Global trade in NHPs includes around 450,000 live individuals and 11,000 body parts (Jiang *et al.,* 2023). Indonesia has recorded 440 transactions involving M. fascicularis, including both live animals and non-lives forms, with total of 117,193 live macaques traded, mainly with the US, from 1990 to 2019 (Sayektiningsih and Broto, 2021).

The spread of infectious diseases from NHPs to human is a growing concern, impacting health risk. Zoonotic transmissions are a significant global health risk, with human-animal contact being major driver of transmission (Sayektiningsih and Broto, 2021). Zoonotic diseases represent 60% of emerging infectious diseases, with 70% of these diseases believed to originate in wild animals (Nijman, 2010; Milbank *et al.,* 2022). While NHP trade is economically profitable in both local and global markets. It also poses threat of disease transmission between NHPs and humans. Tuberculosis is one of such disease frequently reported in NHPs. According to the Center for Disease Control and Prevention (CDC), 0.4% of NHPs in 249 imported shipments from 1990 to 1993 tested positive tuberculosis, which poses a risk to other NHPs and humans working with them (Center for Disease Control, 1993). As a source and potential hub NHPs trade, Indonesia need to enhance its knowledge and preventive measures to manage tuberculosis transmission-related NHP trade and movement. This paperwork will addressed information about zoonotic risk of tuberculosis from NHPs, legislation aspect both international and national level, and preventive measure for NHPs trade-movement for tuberculosis infections.

METHODS

Writing methods of this paperwork were done by reviewing literature such as journal, text book, article, and academic writing from both international and national publisher sources.

RESULTS AND DISCUSSION

In mammals, tuberculosis is caused by members of the *Mycobacterium tuberculosis* complex, which are Gram-positive, acid-fast bacterial rods in the family Mycobacteriaceae. The organisms present in animals include *Mycobacterium bovis* (bovine tuberculosis), *M. caprae* (caprine tuberculosis), *M. pinnipedii*, *M. orgyis*, and *M. microti*. While *M. tuberculosis* and *M. africanum* are primarily maintained in humans, they can occasionally affect animals. The *M. tuberculosis* complex is often considered a single species, with *M. bovis* and *M. caprae* classified as *M. tuberculosis* subsp. *bovis* and *M. tuberculosis* subsp. *caprae* (Spickler, 2019).

Tuberculosis remains one of the most impactful bacterial diseases affecting humanity, with approximately a quarter of all humans infected. It is responsible for a significant number of infection-related deaths and long-term disabilities (WHO, 2020). An estimated 10 million people are infected with TB worldwide, making it one of the top ten causes of human death and the leading cause of death from a single infectious agent. Tuberculosis is a chronic airborne disease that causes high morbidity and mortality in both humans and non-human primates (Pereira *et al.,* 2022).

Some non-human primate (NHP) species may be more or less susceptible to tuberculosis, but all NHPs can develop the disease due to their high genetic similarity to humans. In captive NHPs, infections primarily occur through direct contact with TB-infected humans via inhalation of aerosolized bacteria. Infected captive NHPs exhibit tuberculosis symptoms indistinguishable from those seen in humans and can display the full spectrum of infection outcomes and pathology (Pereira *et al.,* 2022).

Exposure to *Mycobacterium tuberculosis* can result in a wide range of outcomes, from clearance of the pathogen without establishing an infection to active tuberculosis or asymptomatic latent infection. Latent tuberculosis (TB) is a complex state that can reactivate

and lead to active TB. The clinical presentation of tuberculosis is diverse and can affect any organ system, though the lungs are most commonly involved (Pereira *et al.*, 2022).

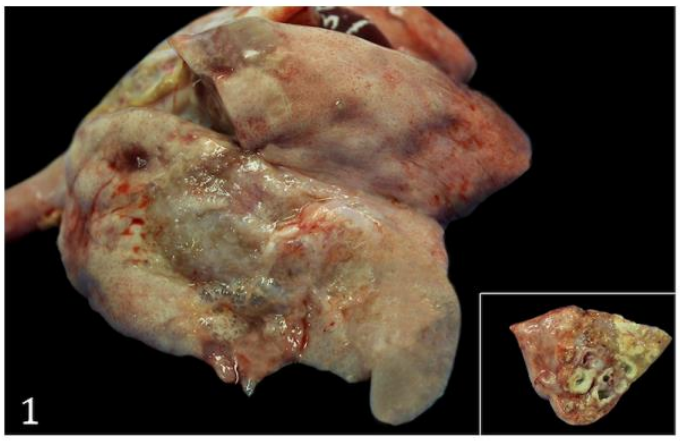

Figure 1. Gross finding chronic tuberculosis in *Macaca mullata* (Source: Pereira *et al.*, 2022).

Clinical symptoms in both humans and NHPs can range from asymptomatic to cough, loss of appetite, weight loss, and potentially death. Transmission from NHPs to humans can occur via coughing or respiratory droplets, with an incubation period ranging from 2 to 12 weeks. In humans, tuberculosis can infect any part of the body, but it most commonly affects the lungs. Tuberculosis is classically characterized by organized inflammatory structures with a necrotic core containing both infected and uninfected macrophages, as well as multinucleated giant cells (Langerhans cells), surrounded by B and T lymphocytes and encased by a rim of fibroblasts. Lesions in active TB can vary widely, even within the same patient, and may be necrotic (caseous), non-necrotizing, or suppurative. Lesions associated with latent TB may be caseous, fibrocalcific, sclerotic, or mineralized (Scanga and Flynn, 2014).

The pathogenesis of tuberculosis in both humans and NHPs typically involves infection via the respiratory tract through inhalation of fine particles containing the bacilli. Transmission may also occur through ingestion, particularly in cases of *M. bovis* infection after consuming contaminated meat. Other potential transmission routes include wounds, tattoo machines, needles, rectal thermometers, nets, and gloves. Once inside cells, the bacteria induce phagocytosis by mononuclear phagocytes, including tissue-resident macrophages and dendritic cells. Successful intracellular killing of mycobacteria enables antigen processing and presentation to T-lymphocytes. T-cells and monocytes are recruited to the infection site by various macrophage-derived cytokines. Mycobacterial products and cytokines released by activated lymphocytes further activate mononuclear phagocytes. Activated macrophages then phagocytize the bacteria and tend to transform into immobile epithelioid cells, forming epithelioid granulomas. Non-resident macrophages and dendritic cells migrate to regional lymph nodes and other tissues; if intracellular killing has not been completed, the infection can progress, and the associated inflammatory response continues. Additional processes contributing to the classical morphology of tuberculosis lesions include the fusion of macrophages to form multinucleated Langhans-type giant cells (Hines *et al.*, 1995; Matz-Rensing and Lowenstine, 2012).

Detecting / Screening Tuberculosis Agent

Detection of tuberculosis can be accomplished by identifying either the causative agent or the immune response to these organisms. Animals that react to either type of test are generally treated as having an active infection. In some species, diagnostic tests may be supplemented with X-rays or other imaging techniques to visualize abnormalities in the internal organs. Several tests available include the tuberculin test, in vitro tests of cell-mediated

immunity, serology, and agent detection by culture and polymerase chain reaction (PCR) (Spickler, 2019). The OIE (World Animal Health Organization) categorizes detection methods for *Mycobacterium tuberculosis* as (1) identification of the agent, (2) delayed hypersensitivity tests, and (3) blood-based laboratory tests (Spickler, 2019; OIE, 2022).

The tuberculin skin test is the primary screening method for cattle and other species and detects cell-mediated immune responses (CMI) or delayed hypersensitivity reactions. Tuberculin, a mixture of bacterial proteins, is injected intradermally, and the site is examined 48-96 hours later for inflammatory swelling. The elapsed time affects the test's sensitivity and specificity. Tuberculin used for livestock contains antigens from *M. bovis* and can also detect infections with other members of the *M. tuberculosis* complex.[9] It is important to note that other members of the *M. tuberculosis* complex, previously considered to be *M. bovis*, are now recognized as distinct species despite identical 16S RNA sequences and over 99.9% genome sequence identity (Spickler, 2019; OIE, 2018).

For primates, the tuberculin test involves injecting 1,500 units (0.1 ml) of undiluted "mammalian old tuberculin" into the edge of the upper eyelid using a sterile 25-27 gauge needle. Tuberculin prepared for human use is generally not potent enough to elicit a response in non-human primates. Purified protein derivatives (PPD) used in bovine tuberculin tests can also be used but are generally considered less sensitive for NHPs. For smaller NHPs, the injection site may be the abdominal skin (OIE, 2022)

Blood-based laboratory tests can enhance detection and confirm or negate the results from intradermal skin tests. There is evidence that an enhanced blood test may follow a skin test during the subsequent weeks, leading to better separation of in vitro blood test responses and greater accuracy. The gamma-interferon assay and lymphocyte proliferation assay measure cellular immunity, while ELISA measures humoral immunity. The gamma-interferon assay detects IFN-γ release from sensitized lymphocytes during a 16-24 hour incubation with specific tuberculin (PPD-tuberculin). The lymphocyte proliferation assay compares reactivity of peripheral blood lymphocytes to PPD from *M. bovis* (PPD-B) and *M. avium* (PPD-A). These tests aim to increase specificity by removing responses to non-specific or cross-reactive antigens from non-pathogenic mycobacterial species. ELISA detects antibodies based on cellular immunity (Spickler, 2019).

Identification of the agent can be done in live animals, as members of the *M. tuberculosis* complex are sometimes found in exudates, biopsy samples from affected tissues, sputum, and other secretions and excretions. Tuberculosis can be confirmed by isolating the causative organism on selective media such as modified Middlebrook 7H10 or 7H11 agar, or Stonebrink's or Löwenstein-Jensen egg-based medium. Some samples may require up to 12 weeks or longer, and these slow-growing organisms can be overgrown by contaminants, so samples should be collected as aseptically as possible. Molecular detection using PCR is another method to identify the agent and can be useful for epidemiological purposes, such as tracing outbreaks (Lecu *et al.*, 2013).

Figure 2. Injection tuberculin (right) and swollen on injected area (left) (Source: Lecu *et al.*, 2013).

Intradermal testing alone is insufficient for a comprehensive diagnosis of tuberculosis. Each method may have risks of false positive and false negative results. Therefore, combining the tuberculin test with an IFN-γ assay or lateral flow assay is advisable to increase sensitivity and specificity. In uncertain cases, it is recommended to apply and critically evaluate a range of methods that rely on different aspects of pathogen detection, including immunology and serology (Lecu *et al.*, 2013).

Medication and Prevention of Tuberculosis

Antibiotics have been used to treat some animals with tuberculosis and this has possibility of clinical improvement without bacteriological cure. Some animals that responded initially later relapse especially patient with inadequate treatment and potential development of drug resistance make some of treatments is controversial and some countries are not permitted. Member of *M. tuberculosis* complex are not susceptible to many common antibiotics (Spickler, 2019).Treatment of tuberculosis in monkey and apes has been undertaken and was successful in some cases. Multidrug treatment may be considered, but only if appropriate isolation and containment facilities can be provided and accurate detection of infection is possible. Biosafety level 3 conditions would be necessary for husbandry of animals under therapy. Treatment in NHP includes the use of multiple drug regimen with isoniazid, rifampin, and ethambutol, or streptomycin and isoniazid over period of least 9-12 months, some of case can up to 30 months. Treatment should be carried with conjunction with culture, clinical examination, and ultrasound (Matz-Rensing and Lowenstine, 2012). Prevention of tuberculosis can be done with routine sanitation and disinfection. Routine test with tuberculin test and other assays periodically will help to find reactor. For workers may poses high risk due to handle NHP should wear protective equipment depend on level of risk (Spickler, 2019).

Case Report Tuberculosis in NHPs

Gross finding tuberculosis in Old and New World monkeys were kept under human care in Rio de Janeiro, Brazil are vary from chronic-active, extrapulmonary, early-activation or latent-reactivation tuberculosis stage.Typical granulomatous were seen in at least one organ. Multiple granulomas were showed in gastrointestinal, lymphatic, musculoskeletal, urinary, and nervous systems. One case with no pulmonary involvement showed many granulomas in the trachea, lymph nodes, mesentery, duodenum, pancreas, liver, and spleen. This research also find that clinical respiratory sign should not considered solely for the clinical of tuberculosis in NHP. Beside coughing as common symptom, weight loss, lethargy, and anorexia were the most clinical sign in NHP with tuberculosis (Pereira *et al.*, 2022).

CDC (1993) reported tuberculosis case from imported NHP with evidence tuberculosis infection was identified in 90 from 20.580 live animal (0,4%) with most of case caused by importation from Mauritius. Investigation also noticed that one NHP facility worker also develop positive tuberculin test after exposure to the infected animal but did not develop active tuberculosis. Tuberculosis detection report was performed from monkey in Weh Island Sabang tourism area with no incident of tuberculosis on NHPs in tourism area (Rahmi *et al.*, 2010).

International Legislation related NHPs trade and movement

International legislation related with NHP trade and movement based on OIE Terrestrial Animal Code Article Chapter 6.12. As state that all NHP species are included in Appendix I and Appendix II of Convention on International Trade in Endangered Species (CITES) may be transported internationally only if accompanied by permit or certificates required under CITES. As most NHP trade and movement are destined for research, educational, and breeding purpose and their sourcing should be in accordance with Article 7.8.7. Before use of NHP, all alternatives to their uses should be explored.

General recommendation for NHP trade are (1) Veterinary Authorities should issue international veterinary certificates only upon presentation of valid CITES document, (2) Veterinary authorities should make sure the animals are individually identified by approved methods to assure traceability and to avoid transmission of diseases, (3) Veterinary authorities of importing countries should not authorize the import of NHP for being kept as a pet, (4) In the case only limited diagnostic testing and NHP sources from a country within natural range of the animal species concern, Veterinary authorities of importing country should place more emphasis on quarantine procedure and less on veterinary certification.

Diagnostic test that subjected to be tested before transportation are for endo/ectoparasite for all species should schedule at least twice with the second test is performed towards the end of quarantine for antiparasitic treatment. Detection tuberculosis should be done at least 3 tests at interval 2-4 weeks for Prosimians, New World Monkey, Old World Monkey, gibbons, and great apes. For tamarins and marmoset should be done 2 tests at interval 2-4 weeks. All methods were used skin test or serology (OIE, 2022).

National Legislation Related NHPs Trade and Movement

National regulation related with NHP trade and movement based on harmonize between Veterinary Authority as describe on Law Number 18 Year 2009 concerning Animal Husbandry and Animal Health as have been amended by Law 41 Year 2014 and Quarantine aspect as describe on Law Number 21 Year 20019 concerning Animal, Fish, and Plants Quarantine. For national trade between area in Republic of Indonesia regulations related with animal trade are Government Decree No. 45 Year 2012 concerning Veterinary Public Health and Animal Welfare and Government Decree No. 47 Year 2014 concerning Prevention and Countermeasures Animal Disease. Implementation strategy for prevention animal disease transmission already stated on Minister of Agriculture Decree No. 17 Year 2023 concerning procedures for controlling movement of animals, animal products, and other carriers of animal disease in area of Republic of Indonesia for primate movement with concern infectious disease agent on rabies.

Legislations related with quarantine measure are Government Decree No. 82 Year 2000 concerning Animal Quarantine and Minister of Agriculture Decree No. 3238 Year 2009 concerning Categorization of Animal Quarantine Pest and Diseases, Categorization and Classification of the Carrier. Both legislations stated quarantine measure for prevention disease and classify for primate's disease concern are rabies, tuberculosis, and Ebola/ green monkey fever. Quarantine measure for prevention risk of entry, exit, and spread of animal pests and diseases are done with quarantine actions including examination, quarantine, observation, treatment, detention, rejection, destruction, and release.

Indonesia Case for Preventive Measure for NHPs trade

Disease prevention measures for non-human primate (NHP) trade and movement, as specified by various international and national legislations, depend on the scale of trade and movement. The required procedures are:

1. **Obtain Documentation from Wildlife Institutions:**
   Secure all necessary documents and recommendations from institutions responsible for the movement of wildlife or endangered animals, such as the Ministry of Environment and Forestry of the Republic of Indonesia.
2. **Obtain Veterinary Authority Documentation:**
   Acquire documents and recommendations from Veterinary Authorities, which address animal disease risk-based measures for both the origin and destination regions or districts.
3. **Animal Health Identification:**

Ensure proper animal health identification and status documentation as required for the issuance of an animal health certificate (SKKH, or Surat Keterangan Kesehatan Hewan). For NHP trade and movement, verify rabies status with a rabies titer check. An animal health certificate will be issued if the rabies titer is at or above 0.5 IU/ml.

4. **Quarantine Procedures:**

Follow quarantine procedures before transporting animals to the destination area. Health screenings will depend on the destination region. According to Government Decree 82 Year 2000, quarantine measures include the examination of documents and animals. Quarantine actions may involve observation and treatment, including screening for rabies and/or tuberculosis (Decree of the Minister of Agriculture No. 3238 of 2009). Rabies screening requires a titer of 0.5 IU/ml or higher, while tuberculosis screening involves a tuberculin skin test, which must yield a negative result. Once all screenings are complete and requirements are met, an animal health certificate can be issued, and animals can be transported to the destination area.

Additional Preventive Measures:

To prevent tuberculosis, individuals working with NHPs—such as veterinarians, zookeepers, quarantine officers, and laboratory workers—should implement preventive measures. These include practicing personal hygiene, applying disinfectants, and using personal protective equipment during the handling of NHPs (CDC, 1993; Matz-Rensing and Lowenstine, 2012).

CONCLUSION

Tuberculosis is a disease caused by the *Mycobacterium tuberculosis* complex, which includes *M. tuberculosis, M. bovis,* and *M. africanum*. These pathogens can infect both humans and non-human primates. Clinical symptoms can vary widely, ranging from asymptomatic cases to coughing, weight loss, and potentially death. Humans are at risk of transmission through the trade and movement of NHPs. Several legislations have been established to prevent tuberculosis transmission on both international and national levels. The OIE Terrestrial Animal Health Code provides international guidelines, while Indonesia's national regulations include Law No. 41 of 2014 and Law No. 21 of 2019. Preventive measures for NHP trade and movement include routine screening using various testing methods, such as tuberculin tests, serological assays, selective media culture, and molecular PCR tests. Additionally, personal hygiene and the use of personal protective equipment are essential during the handling of NHPs.

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