

A report on Tuberculosis in Monkeys (*Macaca mulatta*): A case study at Chittagong Zoo

Rahul Das Talukdar Avi¹, Suman Paul², Samuel Muhit², Md Mongur Morshed Chowdhury³, Arup Sen^{4*}

¹Department of Environmental Sciences, Wageningen University&Research Centre,Netherlands;

²Department of Epidemiology and Public Health, Sylhet Agricultural University, Bangladesh;

³Chittagong Zoo, Bangladesh;

⁴Department of Microbiology and Veterinary Public Health, Chittagong Veterinary and Animal Sciences University, Chittagong, Bangladesh.

Abstract— Simian tuberculosis is one of the most important bacterial diseases of captive monkey in Bangladesh. A prevalence study to characterize *Mycobacterium* infecting tuberculous monkeys in captive managemental systems in Chittagong Zoo was carried out. In the present study, 14 rhesus monkeys which were newly arrived in the zoo and kept in the quarantine were used for the tuberculin skin testing (TST) to determine the prevalence of tuberculosis. An overall of 28.57% (4/14) was recorded by the TST. There were also marked differences in the prevalence of the disease within different age groups. In the tested positive animals, one was died within two days and showed tubercle in the lung and other organs in the post-mortem examination. The lung sample was collected for Zeihl-Neelsen revealed red colored tubercule bacilli. The above examination confirmed that, the macaques were suffering from tuberculosis.

Keywords—*Mycobacterium tuberculosis*, Rhesus monkey, Tuberculosis, Tuberculin, Prevalence.

I. INTRODUCTION

Simian (Primate) tuberculosis (TB) is a major health problem in most of the developing countries. As in many other hosts, simian tuberculosis is also caused by bacterium *Mycobacterium tuberculosis* (Avicenna, 2011). Outbreaks of tuberculosis have been reported in many captive monkey colonies around the world. In Japan, tuberculosis in monkeys has been reported in zoos (Yumi Une, 2007). Many species of *Mycobacteria* can cause disease in primates and other species (Yumi Une, 2007). Recently, two outbreaks of tuberculosis occurred in four different kinds of monkeys and humans were also infected with the disease in Japan (Yumi Une, 2007). In zoos, tuberculosis was reported not only in monkeys but also in several different kinds of

animals (Yumi Une, 2007). TB bacteria can live in the body for years long in non-active form (Moreland, 1970). Primates acquire classic tuberculosis (TB) by contact with other infected nonhuman primates (NHP) or humans through inhalation or the digestive route (Moreland, 1970). It has shown that about 60% of infected NHPs develop Latent Tuberculosis Infection (LTBI); latency is confirmed by a positive tuberculin skin test (TST) (Patel, 2011). This disease and the causative agent *Mycobacterium tuberculosis* have been intensively studied, yet the basis for protection, as well as many of the microbial and immunologic factors that contribute to disease, is not well understood. In Bangladesh outbreak of tuberculosis has been reported in humans and domestic animal but, not yet been reported in captive monkeys. In our study area (Chittagong zoo) outbreak was also not specifically recorded. But it is known that geographically this area is at a risk of tuberculosis, especially in the Chittagong zoo due to the possibility of direct contact of zoo animals with visitors. In our study, we only focused on the prevalence of tuberculosis in Chittagong zoo. The tuberculous monkey is a health hazard especially to other monkeys in the group, but (re)transmission of the infection to humans has been reported as well (Yumi Une, 2007). Due to lack of proper diagnostic and treatment facility TB is increasing and zoonoses transmission occur. In spite of reasonable precautions, outbreaks continue to occur and tuberculosis remains a serious threat to the health of captive monkey and their care takers. To the best of knowledge, there is no published comparative report on the prevalence of tuberculosis in captive monkeys (rhesus monkey) in Bangladesh. Therefore, the following study was carried out with an aim of estimating the prevalence of tuberculosis in captive monkeys (rhesus monkey) with these following objectives:

- 1) To study the prevalence of tuberculosis in captive primates (Rhesus monkey).
- 2) To identify the risk factor associated with the disease.

II. MATERIALS AND METHODS

(1) The survey area: The study was done at Chittagong zoo, Bangladesh.

(2) Study population and study period: In the present study, 14 rhesus monkeys which were newly arrived in the zoo and kept in the quarantine were used to determine the prevalence of tuberculosis. The study time was October 2014 to November 2014.

(3) Study design: A cross-sectional study design was followed in the present study with a view of estimating from the record book.

(4) The capture of Monkey: In the quarantine shed the monkeys were live captured by the caretaker with the use of the net. During the capture, no monkey was injured and all safety measures had taken to check any critical condition. All the monkeys are captured in a humane way.



Fig.1: Capture of the monkey

The original plan was to inject the tuberculin immediately after capture, but the restrictions on entry to two-thirds of the area made the monkey afraid and showed anger. Following the identification of infected animals in the group of the monkey caught, was injected tuberculin and observed the swelling.

(5) Ante-mortem examination and tuberculin test: All the monkeys were included in the sample size were examined physically before they are slaughtered. Age, sex, and weight of the animals were recorded. Additionally, body temperature, pulse rate, respiratory rate, type of nasal discharge (if present), the condition of regional lymph nodes, and visible mucous membranes were examined. Besides purified protein derivative (PPD) tuberculin was injected intradermally to record the swelling above 10 mm for confirmed tuberculosis.

(6) Post-mortem examination: Only one infected monkey has died after two days of injecting tuberculin and through post-mortem of the monkey was done. After post-mortem visual examination of intact organs like kidneys, lung, the liver was done. The tuberculous nodule was found in the visceral organ and grossly diagnosed as tuberculosis.

(7) Laboratory diagnosis: The impression from the tentatively diagnosed TB nodules found in different visceral organs was taken and on glass slide stained following acid fast staining procedures described in Literature review section. Examination of stained smear was carried out in microbiology department of Chittagong veterinary and animal science university.

(8) Data collection: The individual animal identification marking, breed, sex, and age of the animal were recorded. After injecting tuberculin the swelling site was measured at 0 hours and 72 hours in mm.

(9) Data analysis: The recorded raw data were entered into Microsoft excel data base system to be analyzed using Statistical Program for Social Science (SPSS) version 20. Descriptive statistics were computed. Prevalence of tuberculosis was calculated as the number of monkeys found infected with tuberculosis, expressed as the percentage of the total number of monkey examined. Fisher's exact test was used to evaluate the association between the tuberculin positivity and different risk factors. The p-value less than 0.05 (at 5% level of significance) were considered significant in all analyses.

III. RESULTS AND DISCUSSION

Description of the study population:

There were 29 monkeys in the primates quarantine section of the Chittagong zoo. For the study, we captured 14 monkeys which were marked by shaving (Fig.2) different area of the body for identification. Among 14 monkeys 6 were male and 8 female. The average age of male is 1.8 years and female is 2.1 years. Average body weight of the male was 3.02 kg and female 3.45 kg.



Fig.2: Marking for the identification of monkey

Antemortem examination: The diagnosis of simian tuberculosis by clinical examination is of very limited value given that most animals infected with the bacterium do not show clinical signs of the disease and that there are no pathognomic signs of simian tuberculosis in the monkey. Monkey with latent TB is not infectious and may appear healthy for years, but eventual reactivation of latent TB can result in secondary transmission and outbreaks of disease in

established colonies. Reactivation of latent infections that were not detected using traditional screening methods during primary quarantine is emerging as an important factor in the epidemiology of TB in the monkey. Only one monkey was observed emaciated with consistent coughing and the monkey died 2 days after the injection of tuberculin. Besides body temperature, pulse rate, respiratory rate, type of nasal discharge, the condition of regional lymph nodes, and visible mucous membranes were examined.

Intradermal Skin Test: A tuberculin Sensitivity test (TST) was carried out for the diagnosis of M. Tuberculosis in the captive monkey. The sample was provided by the respective authority. For this study, 14 monkeys were live-trapped by using the net. After recording their age, sex the monkeys were then weighed and marking was done. The hair was clipped without traumatizing the skin and the injection site noted. Then an intradermal tuberculin test was performed and injecting intradermally 0.1 ml of tuberculin. Then the swelling of the side was measured (Fig.3). The site was inspected at 24 and 48 and 72-hour interval. In the observation we found 3 monkey's skin swelling above 10 mm. During diagnosis process, a 27 gauge needle was used for intradermal injection of tuberculin. The injecting site was the skin of fore arm region. At first, the animal was captured by the animal caretaker using the net. Then the injecting site was prepared for tuberculin injection. All the monkeys were given 0.1 ml PPD intradermally and then swelling was measured immediately in mm and a wide variety of swelling length was recorded. During the observation, a diseased animal died on the spot. The carcass was subjected to a detailed post-mortem examination and some portions of lesions found were fixed in buffered formalin for histological examination and some are frizzed for microbiological examination. For the microbiological test, the sample was taken in the dept. of microbiology of Chittagong veterinary and animal science university (CVASU) and acid fast was done.



Fig.3: Swelling measurement after 72 hours

Table.1: Prevalence of tuberculosis in captive monkeys (Rhesus monkey):

No. of tested animal	No. of positive case	Prevalence (%) with 95% Confidence interval
14	3	21.42 (-0.07% - 42.91%)

A similar type of skin test was performed in the United States and the considerable animal was found infected (Narasimhan, et.al; 2013). In the study of zoonosis of Non-Human Primates reported 0.5% (9/1621) prevalence of tuberculosis in Rhesus monkey in the USA which is much lower than present study (Narasimhan, et.al;2013). The variation of these two studies was probably due to differences in study design and the number of sample size.

Post Mortem examination:

For determination of the cause of death of the monkey, the postmortem was done on the Post-Mortem room of Chittagong zoo. Before the PM all organs and tissues, including external body, examined in a systematic way (position color, size, weight, shape, consistency, content, smell, an extension of lesions, and aspect of the section). Protective clothing was worn according to bio hazard level of tuberculosis.

The instrument pack also include post-mortem knives, forceps, two scalpel handles (one for cutting, one for burning organ surfaces before taking a microbiology sample), stout scissors (for cutting bones), and fine scissors for dissection.

After finishing the PM disposal of the carcass, appropriate disinfection of self and equipment, to avoid further spread of the disease was ensured.

Post-mortem finding:

The animal was opened aseptically and gross examination of the visceral organ was done. The tubercles had a yellowish appearance and an abscess with necrotic focus and caseation was found. Other findings at post-mortem examination were numerous small focuses usually in the lung, liver, lymph node etc (Fig.4). Tubercles were found in bronchial, mediastinal lymph nodes and after that sample were collected (Fig.5). Tubercle nodule was also found in the lungs, liver, spleen, body cavities and female genitalia as it was chronic tuberculosis. In a study by Francis, (1958) reported the same kind of nodular elevation in lymph node found in the cow. Thus, a tentative diagnosis of TB was made based on the similarities of post-mortem sign found in the dead monkey and the referred study.

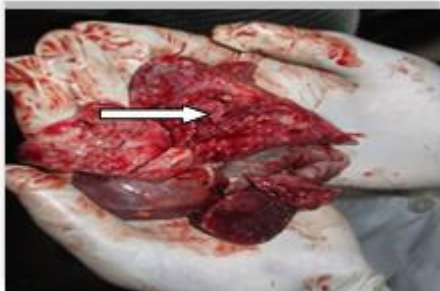


Fig.4: Tubercle nodule in the lung, Liver and Lymph node



Fig.5: Sample collection after post-mortem

colored tubercle bacilli were observed in the lung specimen indicating that the monkeys were exposed to tuberculosis. The cell wall of tubercle bacilli contains (Fig.6) waxy material so it did not take dye at room temperature. But the bacteria took up stain with dye by prolonged application or by heating. When once the bacteria were stained, they resisted decolorization with acid alcohol, but the tissue could be decolorized and took the color of methylene blue. For this reason, bacilli looked red and the tissue looked blue in the acid fast staining result. In a study of Forero, M. et al., (2004), similar identification of red color TB organism was found by Ziehl-Neelsen method for acid-fast staining.

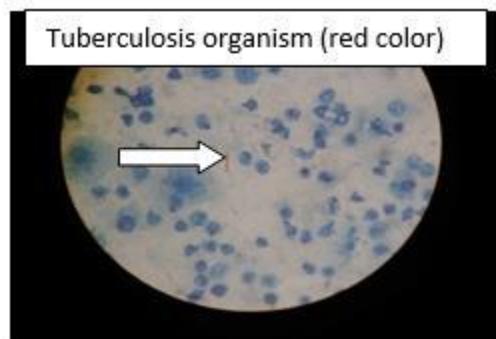


Fig. 6: Tuberculosis organism after acid fast staining

Acid fast finding:

For the study, were made total 10 slides where smear was taken from the lung. Tubercle lesion site was smashed and grinded, then taken a smear from them. After staining, red

Table.2: Risk factors of tuberculosis:

Variable	Category	No. of animal tested	No. of positive animal	Odds Ratio (95% Confidence interval)	P value
Gender	Male	6	2	3.5(.24-51.89)	0.54
	Female	8	1	1	
Body weight	High(≥ 4 kg)	6	3	9.00*(.75-108.3)	0.06
	Low(< 4 kg)	8	0	1	
Age	Adults (≥ 3 years)	5	3	13.33*(1.05-169.56)	0.03**
	Young (< 3 years)	9	0	1	

*Odds ratio was calculated by adding 1 in each cell.

** Significant at 5% level

Variables identified to be significantly associated with status (tuberculosis) in Fisher’s exact test included age, sex, body weight (Table 2). Plasma cells can occasionally be seen in smears of peripheral blood. Hypoalbuminemia and hyperglobulinemia are common findings. Serum protein has the nodulous portentous effects and protein electrophoresis. The organ affected by the lymphosarcoma and upper respiratory distress. Tuberculosis was found to be more prevalent in males as compared to females. Similar gender relationship of tuberculosis was found where the ratio of

female to male tuberculosis cases notified was 1:1.5–2.1 and 70% more smear-positive male than female tuberculosis patients are diagnosed every year and notified to the WHO (Diwan, and Thorson, A., 1999). Adult individuals showed higher prevalence than the young. A strong association ($P < 0.05$) was observed between age and risk of tuberculosis (Table 2). Similar age relationship with tuberculosis was found in human where more than 65 percent of the residents of New York City who died of the disease were over 45 years of age (Robins, 1953). The exposure time probably

increased with increasing age. Thus the older the monkey the higher chance of being TB positive. However, the gender of the animals was not significantly associated ($P>0.05$) with tuberculosis and there was no significant difference between tuberculin positivity and body weight of the monkey.

IV. CONCLUSION

M. tuberculosis is well known to have the widest species range, infecting an extensive range of animals, from cattle to humans, domestic animals to feral or wild ones. In addition, captive animals infected with tuberculosis create problems in the management of zoological collections, increasing the risk of infection to other valuable animals as well as to their keepers. Although tuberculosis in such animals is an important problem, there is a dearth of well-validated data for the diagnosis of the disease in the monkey. Although the epidemiology of simian TB is well understood and effective control and elimination strategies have been known for a long time, the disease is still widely distributed and often neglected in most developing countries. Its public health consequences, although well documented from the past experiences of industrialized countries, have scarcely been investigated and are still largely ignored in these regions. Prevalence (21.42%) of tuberculosis in captive monkeys indicates that the area is at risk of transmission of TB of zoonotic concern. Research is needed to determine when M. tuberculosis is of zoonotic importance and what the underlying mechanisms of transmission are. TB was not possible to find out the possible source of infection and mode of transmission due to the short duration of the study. Further long-term study needed to find out the possible source of infection and specific organism. Outbreaks have severe economic consequences due to animal losses, disruption of research and costs related to disease control. Therefore persons working with monkey should be familiar with the disease and preventive measurements. The increase of TB in such areas calls for stronger intersectoral collaboration between the medical and veterinary professions to assess and evaluate the scale of the problem. Any vaccination research and development program should therefore also take into account the possible application of vaccines to the monkey, particularly in developing countries.

CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

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AUTHOR'S CONTRIBUTION

All authors contributed equally and approved the final manuscript.

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