Animal and Veterinary Sciences
2023; 11(2): 44-51
http://www.sciencepublishinggroup.com/j/avs
doi: 10.11648/j.avs.20231102.13
ISSN: 2328-5842 (Print); ISSN: 2328-5850 (Online)

Tuberculosis Among Lemurs' Captive Breeding at the Parc Botanique et Zoologique de Tsimbazaza (PBZT) in the Madagascar

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To cite this article:

Berthine Razafiarisoa, Christine Razaiarivelo, Roger Ramarokoto, Mbolamanana Ziva Randriaminahy, Mandimby Herivola Randrianarison, Brigitte Annie Bezandry. Tuberculosis Among Lemurs' Captive Breeding at the Parc Botanique et Zoologique de Tsimbazaza (PBZT) in the Madagascar. *Animal and Veterinary Sciences*. Vol. 11, No. 2, 2023, pp. 44-51. doi: 10.11648/j.avs.20231102.13

Received: March 12, 2023; **Accepted**: March 30, 2023; **Published**: April 13, 2023

Abstract: The Madagascar is a country with a high endemic biodiversity. For example, it homes 112 species and subspecies of lemurs with 100% of endemism. They are endangered in their natural habitat due to different threats from anthropogenic pressure and climate change. Between May to September 2022, eight relatives *Varecia varecia substincta* captive breeding at the Parc Botanique et Zoologique de Tsimbazaza deceased in serial and unexpectedly, some of them by tuberculosis. This study is carried out to detect the reasons why these lemurs are died and then, to assess the tuberculosis prevalence among the remain alive lemurs, in objective to proceed in cure. Tuberculosis is a notifiable treatment in the Madagascar. Outcomes has shown that lemurs are predisposed to tuberculosis infection as human primates. It may be present in latent or fatal active forms as miliary tuberculosis. Vulnerabilities depend on gender and species. Females are more fragile than males. Among different species of lemurs, *Varecia variegata subtincta* are the most threatened to tuberculosis followed by *Eulemur fulvus*. Tuberculosis is hereditary and for the first time, its genetic aspects are discovered in the Madagascar. Foremost, genetic predisposition has an influence in individual immune responses. In fact, change in weathers factors has increased underlying individual susceptibilities which led to a serious health problem. Consanguinities are an addition threat for tuberculosis transmission in an endemic island context. These basic fields need further confirmation both for lemurs' conservation and to sustain the control of tuberculosis in the Madagascar.

Keywords: *Eulemur fulvus*, Bioclimatic, Biodiversity, Genetic Predisposition, Lemurs, Miliary Tuberculosis, *Varecia variegata subtincta*

1. Introduction

The Madagascar is assigned among of the 36 hotspot biodiversity countries with a high concentration in endemic species [1]. For example, the country homes 104 species and subspecies of lemurs with 100% of endemism. They are always endangered in their natural habitat due to different threats as anthropogenic pressure and climate change [2]. Moreover, lemurs may be suffered directly or indirectly with common human infectious diseases, either in wild free field [3-5] or in captivity [6]. Tuberculosis (TB) due to *Mycobacterium tuberculosis* is among these infections.

In fact, between May to September 2022, eight (08) relatives *Varecia varecia substincta* deceased in serial and unexpectedly at the PBZT, that some from tuberculosis. Reports about lemurs TB were rare in the Madagascar: a first death is reported in 1969 among breeding ex-situ lemurs at the PBZT [7] then, a death of a pet *Lemur catta* due to a drug-resistant is signalled in 2021, at the south-western of the Madagascar [8].

This design is carried out in the purpose to improve biodiversity conservation in the Madagascar. It is aimed

firstly to identify the reasons why TB is widespread among lemurs' captive breeding and secondly, to lead an opportunistic zoo survey among the remain alive lemurs in objective to assess the TB prevalence and then, to proceed in lemurs' cure: TB is a notifiable disease in the Madagascar. Results are reported in this article.

2. Area, Subjects and Methods

2.1. Area and Period Studies

The PBZT is a national research center, assigned to implement the biodiversity conservation policy of the Malagasy government [9]. It is in charge mostly in the conservation of endemic wild Fauna or Flore species endangered in their natural territories. It serves as a site for mass sustainable environment education too.

The PBZT is located at 1261m altitude in the capital of the Madagascar [10]. It has a tropical altitude climate as Antananarivo city. It covers 07 ha of a current area. Sites area occupancy by studied lemurs are shown in Figure 1. In the objective to have a real dimension of the zone, a topographic map based from Google Earth with collect of Global Positioning System (GPS) points is done to limit the shape file. The treatments used SAS. planet. nightly. 210906.10172 software and geographic information system (GIS) software ArcGIS 10.4.1.

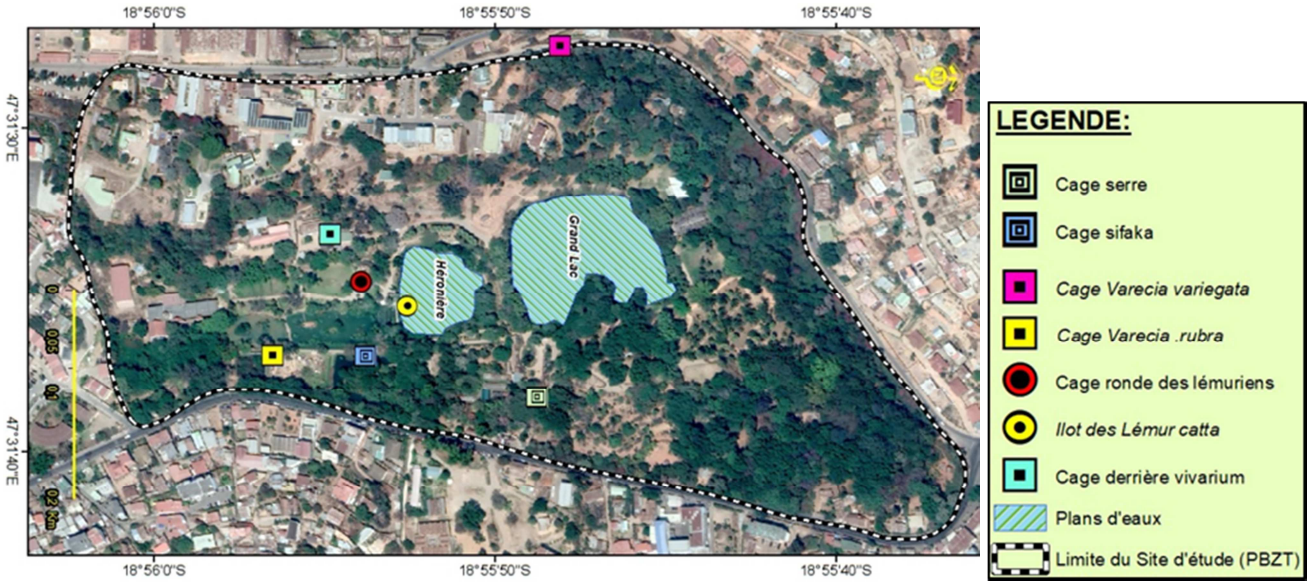

Figure 1. *Sites of lemurs' collection at the PBZT (Source PBZT).*

The period of the studies was between May 2022 to October 2022.

2.2. Methods

Three methods are used in this design: 1) a post mortem method by examination of recorded information from May 2022 to September 2022, 2) a survey method, to assess TB prevalence among the remain alive lemurs, and 3) a TB climate-pathology study, to estimate the probable progress of the disease by taking account weather factors.

Laboratory analysis are always used to confirm the infection by *Mycobacterium tuberculosis* (MTB) and rifampicin resistance (RIF) [11].

2.2.1. Post Mortem Method

Collected informations recorded from *Varecia varecia subtincta* are: 1) date of arrival at the zoo, 2) origin (wild or captive-born), 3) sex (male or female), i) affiliation, iii) date of birth, iv) date of death, v) clinical observations or autopsy results and laboratory analyze results for suspected organ samples (liver, lungs,...)[i]. These data aimed to identify the reasons of deaths of the eight *Varecia varecia subtincta* from May to September 2022.

2.2.2. Survey Method

A mass screening survey is done from September 2022 to October 2022 among the remain alive lemurs' captive breeding. The assessment used lemurs gastric samples approaches with GeneXpert MTB/Rif test, to detect simultaneously *Mycobacterium tuberculosis* infection and a rifampicin resistance (RIF)[ii]. TB positive infections are classed: trace, very low, high, very high.

2.2.3. Climate-Pathology Study

Many studies in the world are reported the impact of the climate seasonal variations on the incidence of the tuberculosis diseases [12-15]. Time-series analysis showed mainly the high correlation of three climatic parameters: temperature, humidity and rainfall, on TB incidence [16].

Informations about these weather factors (temperature, humidity and rainfall) are collected from the national meteorology data for the Antananarivo station. The aims are to take into account the impact of climate factors in lemurs TB incidence. For this, weather factors values are collected from January 2022 to September 2022 then, compared with

the normal climatic standards values[iii] to appraise the change.

Moreover, Humidity Index (HI) is estimated from temperature and air humidity parameters. Mussidan France method is used to define organism discomfort [17]. Discomfort sensations appear when Humidex value is 30 and increases with it.

3. Results

3.1. Global Characteristics of the Studied Lemurs

Globally, thirteen species of lemuridae is enrolled in the study: seventy-four females and sixty-two males. All the lemurs are wild endemic species (Table 1).

Table 1. *Lemurs characteristics.*

N	Scientific name	Vernacular name	IUCN status [18]	Species number
1	*Eulemur albifrons*	White-fronted brown lemur	Rare (R)	5
2	*Eulemur albocollaris*	White-collared brown lemur	Vulnerable	1
3	*Eulemur Coronatus*	Crowned lemur	Endangered	25
4	*Eulemur flavifrons*	Blue-eyed Black Lemur	Endangered,	4
5	*Eulemur fulvus*	Brown lemur	Rare (R)	26
6	*Eulemur macaco*	Black Lemur	Vulnerable	11
7	*Eulemeur mongoz*	Mongoose lemur	Endangered	3
8	*Eulemur rubriventer*	Red-bellied lemur	Vulnerable	16
9	*Eulemur rufus*	Red fronted lemur	Rare	20
10	*Hapalemur griseus*	Grey Gentle Lemur	Critically Endangered	2
11	*Lemur catta*	Ring-tailed lemur	Endangered	12
12	*Varecia variegata subtincta*	Black and White Ruffed Lemur	Endangered	08 (all dead)
13	*Varecia variegata editorium*		Endangered	3
Total				136

The criteria for threat Status of IUCN are: i) Endangered (E) when the taxa are in danger of extinction and the survival is doubtful, ii) Vulnerable (V): taxa believed likely to move into the "Endangered" category in the near future, iii) Rare (R), taxa with small world populations that are not at present "Endangered" or "Vulnerable", but are at risk if the causal factors continue to operate. According IUCN list, seven (07) of the species are classed as “endangered” of extinction, three (03) “vulnerable” and three (03) “rare”.

3.2. Varecia variegata subtincta and Tuberculosis

Varecia variegata subtincta (Figure 2) is listed “Endangered” by IUCN criteria. The subspecies is found in the eastern rain forest of the island. Diets in wild field are mostly fruit and legumes and are supplemented with small amounts of leaves. Females are offspring two or three infants per year.

Figure 2. *Varecia variegata subtincta (Source PBZT).*

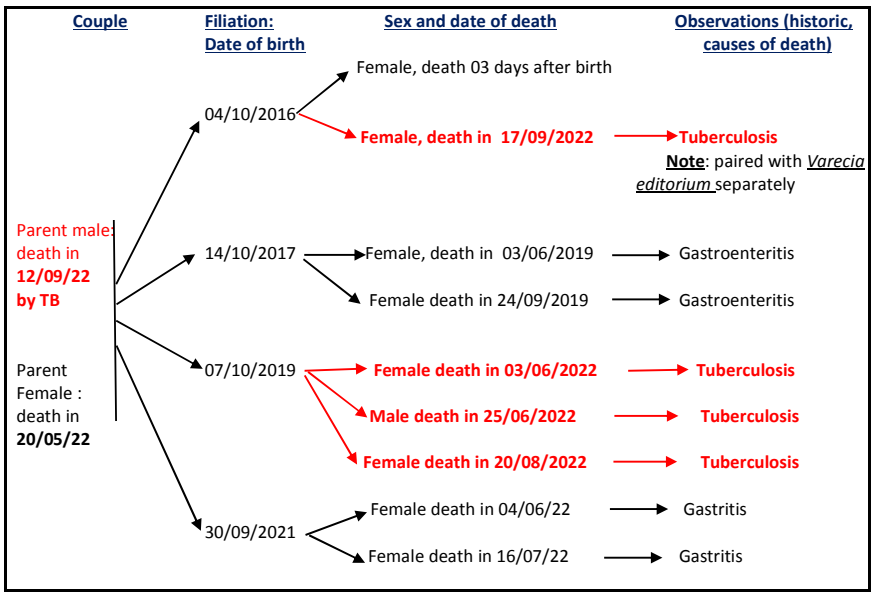

Figure 3. *Varecia variegata subtincta filiation and TB.*

The parent male of *Varecia variegata subtincta* came from wild habitat in 2008 and the female was captive born in 2007. They have given nine (09) captive-born offspring: a triplet (2019) and twins in each years: 2016, 2017 and 2021. Seven (07) of the group were lived in the same box before their death. The first female born in 2016 is paired in another cage with a male *Varecia variegata editorium.*

All the family of the black-and white ruffed lemurs (number= 8) were dead from May 2022 to September 2022. Female parent died firstly in May 2022 and lastly the parent male in September 2022. Parents male and four of the issues were dead by miliary tuberculosis and for the others descendants, by digestive diseases (Figure 3).

Autopsy examinations of suspected organs have shown a wide spread of white nodules lesions throughout different organs (livers, kidneys, lungs) (Figure 4): results of the laboratory analysis confirmed miliary tuberculosis case.

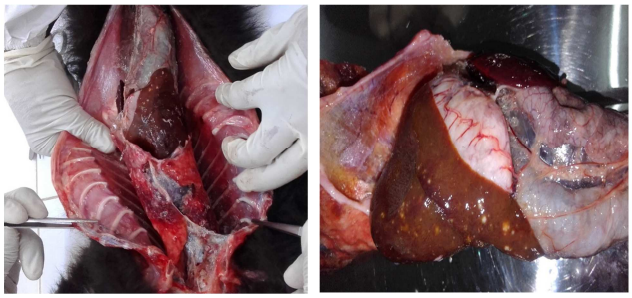

Figure 4. *Miliary TB infection of Varecia varecia variegata organs (Source PBZT).*

3.3. Remains Lemurs and Tuberculosis

Among the remains lemurs (number = 128), thirteen point twenty-eight percent (17/128) are suffering by latent form of TB: average two-thirds (62,5%) by TB trace and the others by TB very low form. Six species have especially TB infection. There is not a big difference between gender. However, *Eulemur fulvus* is the most species infected by latent TB (47,05%) (Table 2).

Table 2. *Distribution of TB among remains lemur species.*

Species	Distribution	Number
Eulemur Fulvus	4= trace (3F; 1M); 4=very low (1F; 3M)	8
Eulemur coronatus	2=trace (F)	2
Varecia editorium	2= trace (M)	2
Lemur Catta	1=trace (F); 1=very low (F)	2
Eulemur Rufus	2= very low (M)	2
Eulemur Flavifrons	1= trace (F)	1
Total (9 F; 8M) (F= Female; M= Male)		17

Eulemur fulvus or brown lemur is the most widespread of the diurnal lemurs in the Madagascar. It has one issue per year. Its diet consists in fruit, leaves and flowers. It breeds well in captivity (Figure 5).

Figure 5. *Eulemur fulvus (Source PBZT).*

Parents are captive breeding and coupled in 2005. They have twenty-five first generation posterities with seven (07) descendants infected by TB: three (03) by trace form and four (04) with very low form.

First generation male and female of *Eulemur fulvus,* both infected by a very low TB, has given birth to a more serious low TB female (Figure 6).

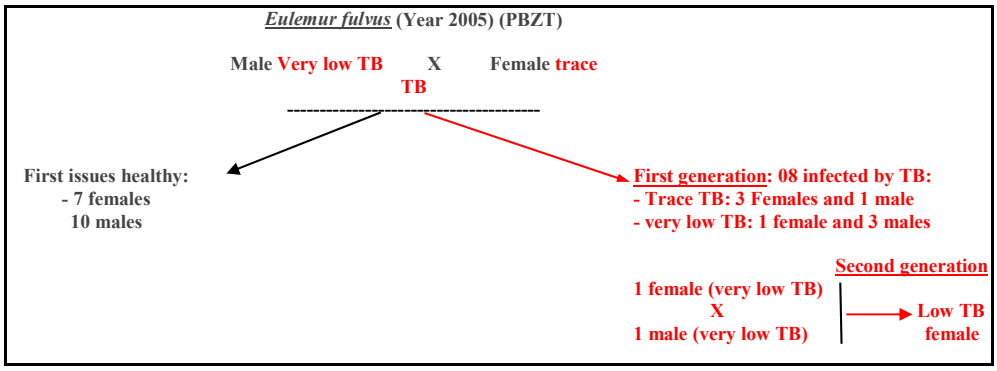

Figure 6. *Eulemur fulvus filiation and TB.*

3.4. Climate Pathology of Tuberculosis

There are two distinct seasons at PBZT: a first season from October to March which is hot and humid period, followed by a second season from April to September which is cold and dry.

3.4.1. Temperature Factors

Coldest months are from June to September where temperature minima varied from 10 to 11°C and the hottest months are from October to March, with temperature maxima 25 to 26°C (Source: normal temperature for Antananarivo city). During the study period, temperature maxima varied from 21,6 to 26°C with maxima in March (26,8°C) and the minima value is in July (18,6°C). Figure 7 highlights monthly temperature variations from January to September 2022.

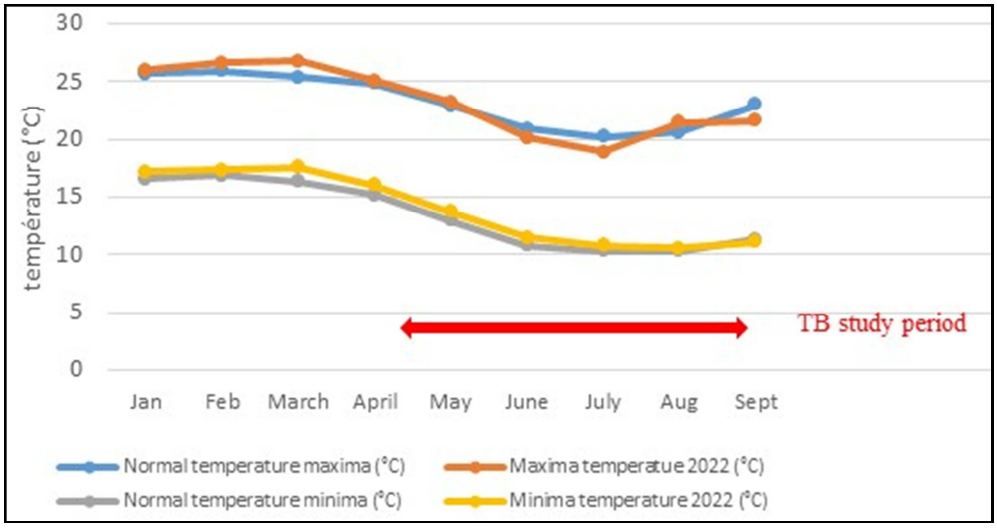

Figure 7. *Monthly temperature variations (°C) at PBZT compared with normal temperature (Source: National Meteorology office).*

3.4.2. Precipitations Factors

Rainfall concerns the distribution of precipitation in the space and in the time. During the study period, the rainiest month is in December with a greater value than normal: 300 mm (normal 388.9 mm). From April to September, the rainfalls are very low, between 0.1 mm to 2.8 mm (normal: 7mm to 20mm).

Monthly rainfalls are irregular from January to September 2022. Precipitations are higher in January (366 mm), March (234,6mm) and lower in April (7,6 mm) compared with normal precipitations for these months (respectively 270 mm, 183 mm and 51 mm). It is lower than normal for the others months. Figure 8 shows the rainfall variation for 2022 from January to September.

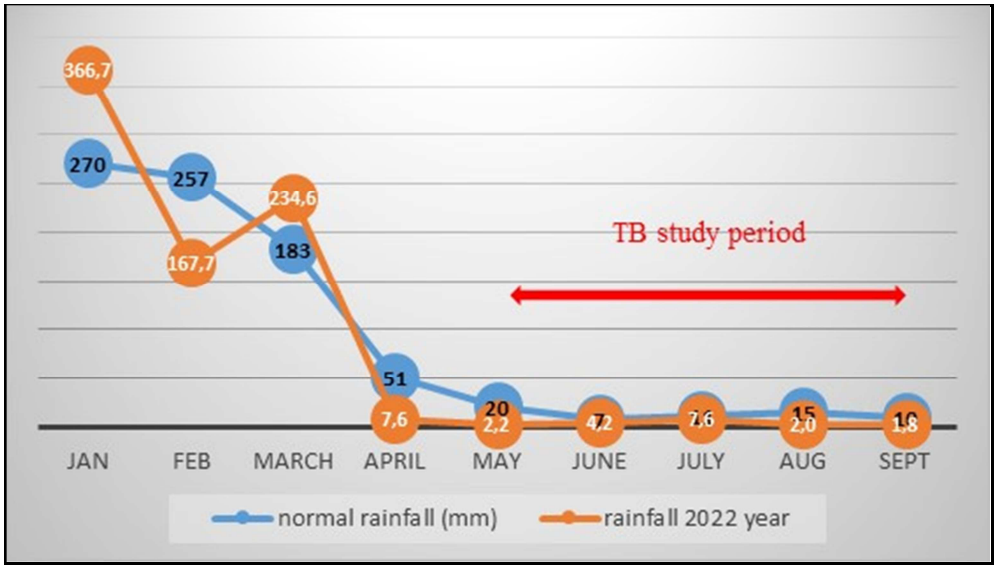

Figure 8. *Monthly rainfall variations (mm) from January to September 2022 compared with normal rainfall (Source: National Meteorology office).*

3.4.3. Humidity Factors

Humidity is defined as the amount of water vapour in the air. When humidity is high, there is a lot of moisture in the air and consequently, the wetter is fell outside. During the study period, air humidity is almost saturated (93 to 98%) compared with the normal humidity values that prevails locally between January to September 2022 (72 to 81%). Figure 9 indicates humidity deviations for the 2022 year at the PBZT.

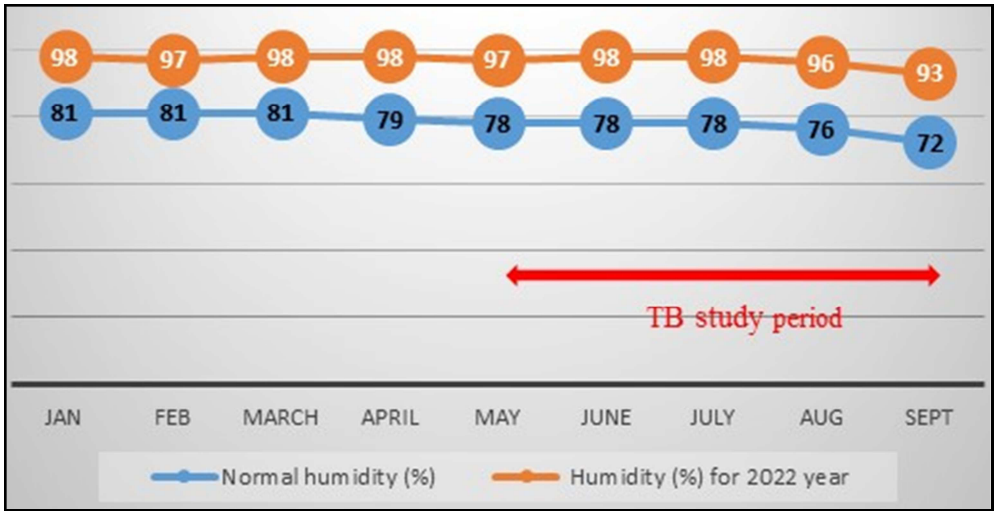

Figure 9. *Monthly humidity variation (%) from January to September 2022 compared with normal humidity (Source: National Meteorology office).*

4. Discussions

4.1. Endemic TB Context

Tuberculosis is a basic airborne infectious disease due to *Mycobacterium tuberculosis*. The contamination occurs mainly by inhaling microscopic droplets dropped by a sick person into the air by coughing, expectorating or sneezing. *Mycobacterium tuberculosis* may resist for a long time in the environment and can be transmitted easily in favourable conditions as immune deficiencies, favourable genetic ground, stress or climatic change.

TB is a progressive chronic infection with latent asymptomatic form that may change in active form. Usually, the disease affects the lungs but may involve in any organ when the *Mycobacterium tuberculosis* are disseminated through a pulmonary vein and produce a small tubercles bacilli likeness numerous millet seeds in size (1 to 2 mm) hence, the name miliary TB. This last form is complicated and occurs in 1-3% cases. It is always lethal [19, 20].

TB is ever a major public health problem in the Madagascar. Since 1964, the island was among countries that benefited of regular support from WHO for a national tuberculosis control program [21].

At the same period, three (03) lemurs were dead by TB among the wildlife animals' captive breeding at the PBZT: one of them in 1964 and the two others in 1966.

Since 2019, tuberculosis is stated as a global public health emergency in the Madagascar. Then, the island is listed among the global "high burden country" (HBC) for tuberculosis with an epidemiological incidence of 100 000 populations per year [22].

4.2. Genetic Tuberculosis for Lemurs

Tuberculosis may be present in active or latent form among the lemuridae family.

Varecia variegata subtincta species are the most exposed to the primary form. The latent form is more prevalent among the others species even it is more dominant among *Eulemur fulvus* species. Miliary TB is fatal for 45,4% (5/11) of the *Varecia variegata subtincta* family and the TB morbidity rate is 32% (8/25) among *Eulemur fulvus* descendants. In a same history exposure, frequency of TB cases among monozygotic twins' human are 69.2% and the rate is 26,3% among dizygotic co-twins [23]. All dizygotic co-twins *Varecia variegata subtincta* (triplets) are dead, indicating a greater fragility of the species for TB diseases.

However, TB is highly hereditary among lemurs mainly for *Varecia variegata subtincta* and *Eulemur fulvus* species. Otherwise, it is well established in human that under the same conditions of exposure to *Mycobacterium tuberculosis,* there is evident relation between immunity-related genes and TB susceptibilities [24, 25]. Moreover, TB among the second generation of lemurs confirmed the inborn gene transmission: twin male issues from female *Varecia varecia variegata* infected by miliary TB and a safe male *Varecia variegate editorium* are infected by TB trace. Then, a couple of first generation of *Eulemur fulvus* both infected by a very low TB has given birth to a more serious low TB female: consanguinity is an addition risk factor in susceptibility to TB infection [26].

At least, if TB frequency is distributed among two sexes for *Eulemur fulvus*, female *Varecia variegata subtincta* are more exposed to miliary TB. It would be interesting to confirm the possible genetic predisposition of lemurs to develop tuberculosis disease in endemic context to improve their conservation.

4.3. Bioclimatic of Tuberculosis

During the period of the successive death of *Varecia variegate subtincta*, there is not a great variation in temperature parameters. In contrast, monthly rainfalls are disturbed and wetness is very high almost along the study period, compared with the normal values.

Moreover, in May 2022, the interaction of temperature (23°C) and relative humidity (98%) has affected directly the well-being and the health of lemurs. Humidex value equivalent to 32 has leads to body stress and generates discomfort to the organism.

Correlation with meteorological factors and TB infection are evident. In context were lemurs' enclosures are far enough apart to avoid contact groups and animals' keepers were TB safe, high abnormal humidity levels has aggravated life conditions and led to discomfort organism, favourable to TB development.

In previous studies, it is established that: i) high precipitation and relative humidity of the previous month increased TB infections [27], ii) if the temperature influence TB transmission, humidity led in reactivation of latent TB to active form [16] and at least, iii) relative humidity is in favour of *Mycobacterium tuberculosis* development which consequently, permits progression of tuberculosis from latent infection to active stage [28]. Outcomes of the studies are in agreement with these assertions.

In the Madagascar, airborne diseases are among the health climate risks challenge. Country strategies include meteorological climate information with early warning airborne disease monitoring [29]. Climate change impact on the resurgence of tuberculosis is evident.

5. Conclusion

In TB endemic context, non-human endemic primates are predisposed to the disease too. TB is present in lemuridea family in the latent or active fatal forms. Vulnerabilities depend on species and gender. In a same living conditions, some endemic species of lemurs are prone to TB: *Varecia variegata subtincta* is the most threatened species with a fatal active form, followed by *Eulemur fulvus* in latent form. Females are more fragile than males.

Moreover, climate change is a real threat for the wildlife biodiversity's survival. Modification in weathers factors has increased underlying individual TB susceptibilities that leads to a serious health problem.

For the first time, TB genetic aspects are revealed in the Madagascar. Genetic predisposition is foremost in tuberculosis pathogenesis. It impacts individual immune responses. Outcomes would help to prevent tuberculosis transmission and would serve a guide for an appropriate conservation strategy by taking into account the genetic predisposition in endemic TB and climate change contexts.

Finally, the consanguinity is an addition risk for TB transmission more than anthropogenic pressures, destruction of the natural habitat and climate change. Basic fields of research are needed to improve lemurs' conservation and would help to sustain the control of tuberculosis in the Madagascar.

Acknowledgements

We are grateful to IPM and PNLTB technicians for their help in laboratory measurements and to all Fauna PBZT teams for their support during the study.

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ii National TB Program

iii climate normals are calculated from 30-year averages for climate variables like temperature and precipitation