

## Diagnóstico de Tuberculosis en primates no humanos a través de prueba intradérmica en Centros de Fauna Silvestre de la Ciudad de Guayaquil, Ecuador

### Diagnosis of tuberculosis in non-human primates through intradermal testing in wildlife centers in the city of Guayaquil, Ecuador

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**Abstract:** Tuberculosis is a zoonotic disease of public health danger. Non-human primates are a susceptible group, being the objective of the present investigation to determine positive cases of tuberculosis and to correlate the risk factors with the degrees of reaction presented. Intradermal tuberculin was the test of choice in the present study, oriented under an experimental intention, quantitative approach, applied type. The total population of non-human primates in captivity in two Wild Animal Rescue Centers in the city of Guayaquil was examined by inoculating 13 specimens with 0.1 ml of intradermal Bovine PPD Tuberculin in the right eyelid. Subsequently, the degrees of postinoculation reaction were evaluated under a criterion of 0 to 5 during 24, 48 and 72 hours. The result was 0% of positive cases detected, with grade 1 reaction in two primates, attributing the reaction according to the clinical history, to the close human-animal contact as a possible risk factor. Thus concluding that there is no difference between the risk factors with respect to the reactions presented by the primates, however, there is a difference in the number of reactions in relation to the registered primates.

**Keywords:** Tuberculosis, non-human primates, Diagnosis, Intradermal Tests, Wildlife.

**Resumen:** La tuberculosis es una enfermedad zoonótica de peligro para la salud pública. Los primates no humanos son un grupo susceptible, siendo el objetivo de la presente investigación el determinar casos positivos a tuberculosis y correlacionar los factores de riesgo con los grados de reacción presentados. La tuberculina intradérmica fue la prueba a elección en el presente estudio, orientado bajo una intención experimental, de enfoque cuantitativo, de tipo aplicado. Se examinó la población total de primates no humanos en condiciones de cautiverio en dos Centros de Rescate de Animales

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Silvestres en la Ciudad de Guayaquil, inoculando a 13 especímenes con 0.1 ml de Tuberculina PPD Bovina intradérmica en el párpado derecho. Posteriormente, se evaluó los grados de reacción postinoculación, bajo un criterio de 0 a 5 durante las 24, 48 y 72 horas. Resultando 0 % de casos positivos detectados, con reacción grado 1 en dos primates, atribuyendo la reacción según el historial clínico, al estrecho contacto humano- animal como posible factor de riesgo. Concluyendo así que no hay diferencia entre los factores de riesgo respecto a las reacciones que presentaron los primates, sin embargo, si hay diferencia en la cantidad de reacciones en relación con los primates registrados.

**Palabras clave:** Tuberculosis, primates no humanos, Diagnóstico, Pruebas Intradérmicas, Fauna Silvestre

## Introduction

Tuberculosis is a zoonotic disease with a high epidemiological risk because it is one of the infections with the highest mortality rate in the world. In the year 2022, it is estimated that 1.3 million people died from tuberculosis worldwide (WHO, 2023). A member of the Mycobacterium group is the cause of this disease that affects humans and a wide variety of animals, both domestic and wild, and it is this human-animal relationship that increases the risk of spreading tuberculosis.

In Ecuador, 6 094 cases of susceptible tuberculosis were registered in 2018, with an incidence rate of 34.53 per 100 000 inhabitants. The province of Guayas experienced the highest concurrence, with 3 354 cases, representing 55.03 % of the total (Ministry of Public Health, 2018). To this is added, poor management in treatment and drug resistance, which do not allow the success of tuberculosis control programs (Trivedi et al., 2012, Howie et al., 2021). In the group of wild animals, non-human primates have been a point of interest in studies of zoonotic diseases, because they are intermediate hosts and reservoirs of a variety of infectious agents. The risk of infection of non-human primates with tuberculosis increases when in contact with humans and domestic animals, since the habitat of this primate species is located in countries with the highest rates of this disease in the world. However, cases have been reported in captive species, with zoos being a point of exposure between wild species and humans (Namasivayam et al., 2019). Currently, accurate diagnosis of tuberculosis in non-human primate colonies remains a challenge. The tuberculin skin test is known as the

standard screening tool for the diagnosis of tuberculosis infection (Bonovska et al., 2005; Gong et al., 2017). Despite the questioning in its results due to false positives or false negatives, it is still a very good diagnostic validity tool (Bonovska et al., 2005; Gong et al., 2017). Of the 21 existing species of primates in the country (Ministry of Environment, 2019), there were no data on the evaluation of the presence of tuberculosis in these animals, under this lack of information the results presented in this article, fulfills the function of keeping a record of control of cases of the disease in this species. Tuberculinization method in non-human primates The tuberculin test in primates is based on the use of bovine PPD. This is produced from PPD (Purified Protein Derivative), made from filtered protein extracts of *Mycobacterium bovis*. A dose of 0.1 ml is administered by intradermal injection in the eyelid of the animal, with a concentration of 20,000 IU/ml of bovine PPD (Proaño-Pérez et al., 2006).

To perform the test, it is recommended to depilate the area of 5 to 6 cm<sup>2</sup> and preferably that the same professional who performed the tests is the one who maintains an adequate follow-up of the symptoms through touch, observation and measurement of the induration in the following 24, 48 and 72 hours (Cely et al., 2011). To consider whether the animal is positive to the tuberculin test, the size of the protrusion formed must be equal or greater than 5 mm. If the animal is positive, it should be isolated and the result confirmed, since the test performed is only considered as a recognition test (Albornoz Villacrés, 2012).

### **Metodología**

The present methodology was oriented by an experimental intention, of applied type, whose objectives are descriptive and using experimental variables, in a diachronic exercise considering that the species are under observation, so the environment is controlled in a laboratory of the wildlife center of Guayaquil, Ecuador, taking as reference what the author Von Pirquet proposes, on the diagnosis of tuberculosis, thus our scopes in this research were quantitative as we sought to identify the

relevance of the test and in this way the method that was followed maintained an inductive logic. In the last reviews of non-human primates, it was empirically observed through the recording of their behaviors that these species have greater possibilities of contracting tuberculosis derived from these behaviors, so the detection of tuberculosis is fundamental to ensure their health within the Fauna Centers in question. Based on the above, the methodological approach is to test the recommendations made by Nagar et al (2006) on the correct way to diagnose the pathology and thus prevent contagion. In this sense, the approach followed was oriented by methodological inputs that allowed assuring a rigorous traceability route on the environmental conditions that promote non-human primates to contract Tuberculosis and the way in which the intradermal test is effective for its detection. The research was developed with the total population of non-human primates in captivity conditions in two Wild Animal Rescue Centers in the city of Guayaquil, Fundación Proyecto Sasha and Narayana, inoculating each specimen with 0.1 ml of Bovine PPD Tuberculin intradermally in the right eyelid. Subsequently, the local palpebral reaction was evaluated at 24, 48 and 72 hours, according to the assessment criteria in Table 1.

**Table 1.** *Intradermal tuberculin: palpebral assessment*

Grado	Reaction
0	No reaction (SR)
1	Hematoma-extravasation of blood in the eyelid (H)
2	Varying degrees of palpebral erythema (E)
3	Moderate swelling with or without erythema (TM)
4	Evident eyelid swelling with drooping and with or without erythema (TM)
5	Palpebral necrosis, variable degrees of swelling and partial or complete closure of the eyelid (NP).

Fuente: WOA (2022)

Based on the above criteria, the interpretation determines that grades 1 and 2 are considered as negative, grade 3 as inconclusive and grades 4 and 5 are interpreted as positive.

## Resultados

The results obtained through the inoculation of Intradermal Bovine PPD Tuberculin in this study showed that of the 13 non-human primates evaluated, 100% were negative to the test. In Table 2, it can be observed that, between the months of November to December of 2023 and January of 2024, the results were negative.

December 2023 and January 2024, a total population of 13 non-human primates were studied, of which 7 belonged to the Sacha Project Foundation and 6 to Narayana. According to their species, they were grouped into: *Saguinus fuscicollis* (8 % 1/13), *Cebus aequatorialis* (46 % 6/13), *Sapajus apella* (15 % 2/13), *Saimiri cassiquiarensis* (8 % 1/13), *Alouatta palliata* (15 % 2/13) and *Ateles geoffroyi* (8 % 1/13).

**Table 2** *Distribution of non-human primates according to species, sex and age in relation to the wildlife center*

	Fundación				
	Proyecto Sacha	Narayana	TOTAL	FR	
Especies	<i>Saguinus fuscicollis</i>	1	0	1	8%
	<i>Cebus aequatorialis</i>	2	4	6	46%
	<i>Sapajus apella</i>	1	1	2	15%
	<i>Saimiri cassiquiarensis</i>	1	0	1	8%
	<i>Alouatta palliata</i>	2	0	2	15%

	Ateles geoffroyi	0	1	1	8%
	Especímenes	7	6	13	100%
Sexo	Hembras	3	1	4	31%
	Machos	4	5	9	69%
Edad	Juvenil	5	1	6	46%
	Adulto	2	4	6	46%
	Geronte	0	1	1	8%

The postinoculation evaluations carried out at the first 24, 48 and 72 hours, determined 100% of the population as negative, presenting 2 individuals with grade 1 reaction corresponding to the presentation of hematoma or blood extravasation in the eyelid, which is detailed in Table 3. Likewise, information corresponding to each wildlife center regarding the origin, habitat and health plan of each of the non-human primates evaluated, in order to identify the possible risk factors that could influence or not the diagnosis of tuberculosis.

**Table 3** Results of tuberculinization during 24, 48 and 72h in relation to risk factors.

		R24H+	R24H-	R48H+	R48H-	R72H+	R72H-	TOTAL R+
Centro	Proyecto Sacha	2	5	1	6	0	7	3
	Narayana	0	6	0	6	0	6	0
Lugar de origen	Naranjal	0	2	0	2	0	2	0
	Milagro	1	2	1	2	0	3	2
	Bucay	0	1	0	1	0	1	0
	Cañar	1	0	0	1	0	1	1
	Sin registro	0	5	0	5	0	5	0

		Nacido en el centro	0	1	0	1	0	1	0
Contacto con Comparte jaula	Si	2	6	1	7	0	8	3	
	No	0	5	0	5	0	5	0	
Contacto con Humanos	Si	2	11	1	12	0	13	3	
	No	0	0	0	0	0	0	0	
Contacto con otras especies	Si	2	11	1	12	0	13	3	
	No	0	0	0	0	0	0	0	
Cuarentena	Si	2	11	1	12	0	13	3	
	No	0	0	0	0	0	0	0	
Vacuna	Si	0	0	0	0	0	0	0	
	No	2	11	1	12	0	13	3	

R(n)H+: If it presented reaction at n (24, 48 or 72) hours.

R(n)H-: No reaction at n (24, 48 or 72) hours.

According to the records in the table, in relation to the place of origin, there were 3 grade 1 reactions, 2/3 (67%) correspond to a primate from the Milagro canton during the first 24 and 48 hours respectively and 1/3 (33%) reactions during the first 24 hours in a primate from the province of Cañar. When relating the grades with the presence or absence of cage companions, it was obtained that the 3 (100 %) reactions registered during the first 24h and 48h after tuberculinization in 2/13 primates

were living in cages shared with primates of the same species. To evaluate the habitat from the shared cage variable, 8 shared (62 %) cages with the same species, while 5 (38 %) did not. Relating this data with the degree of reaction, it was determined that the 3 (100 %) reactions registered during the first 24h and 48h later, in 2/13 primates were living in cages shared with primates of the same species.

Regarding the people who come in direct contact with the primates studied, either for feeding, cleaning, medical attention or cage maintenance. It was obtained as a result that the first group of primates 5/13 (38 %) registered approximately a number of 10 people who had direct contact with them, the second group 2/13 (15 %) had direct contact with approximately 5 people daily and the third group that were 6/13 (46 %) had contact with 3 people. When establishing a relationship between the degrees of reaction presented, it is established that 3/3 registered reactions (100%) were reflected in primates that were in daily contact with more than 10 persons per day.

When analyzing whether the primates studied were close to other species in relation to their cages, 6/13 (46%) were close to birds and goats, 2/13 (15%) were close to birds, felines and canines, and 5/13 (38%) were close to birds, reptiles and canines, i.e. 100% were close to other species.

Regarding sanitary management, all individuals (100 %) registered having been subjected to quarantine during the first days prior to their introduction in each center; however, no primate (0 %) was vaccinated as a prevention method for tuberculosis.

Table 4 shows the analysis of variance to establish the correlation between the degrees of reaction and the risk factors, obtaining the following results.

**Table 4** *Summary of mean and variance between risk factors and reactions to the intradermal tuberculin test.*

RESUMEN	Cuenta	Suma	Promedio	Varianza
Contacto con humanos	2	13	6,5	40,5
Comparte jaula	2	13	6,5	40,5

Vacuna	2	13	6,5	84,5
Cuarentena	2	13	6,5	40,5
Reaccionó	4	6	1,5	1
No reaccionó	4	46	11,5	1

**Table 5** Analysis of variance between risk factors and intradermal tuberculin test reactions.

ANÁLISIS DE VARIANZA						
Origen de las variaciones	Suma de cuadrados	Grados de libertad	Promedio de los cuadrados	F	Probabilidad	Valor crítico para F
Filas	0	3	0	0	1	9,27662815
Columnas	200	1	200	100	0,0021284	10,1279645
Error	6	3	2			
Total	206	7				

A p value of 1 ( $> 0.05$ ) was obtained, so the null hypothesis is accepted, that is, there is no difference between the risk factors with respect to the reactions presented by the primates, however, a second p value of 0.002 ( $< 0.05$ ) was obtained, so it is deduced that there is a difference in the number of reactions in relation to the registered primates (Table 5). At present, there are not enough studies in Ecuador to evaluate the situation of tuberculosis in wildlife and therefore in non-human primates. The data obtained in the present investigation generate new information regarding the disease, obtaining 0 % of positive cases for tuberculosis in the primates under study. However, in the research of Albornoz (2012), he detected the presence in 2 out of 25 primates, carried out in the Guayabamba Zoo in Quito, reflecting 12 % of positivity for tuberculosis by means of the tuberculin test. There are studies in Colombia, carried out by Estrada (2011) where the same

technique was used, under the same parameters, obtaining the same results, 0 % of positive cases. It is important to note that each data is added to give a reference of the status of the primates and the current condition regarding the presence of tuberculosis. In a study conducted by Obaldía, et al. (2018) reported an outbreak of tuberculosis in a colony of Aotus primates in Panama, using antibody release tests and gamma interferon for diagnosis. In their epidemiological analysis, they confirmed that the outbreak was the result of exposure to the disease, ensuring that humans were the most likely source of transmission, a conclusion that supports the results of the present study that identified direct human-nonhuman primate contact as the main risk factor in the wildlife centers evaluated. Currently, accurate diagnosis of tuberculosis in nonhuman primates remains a challenge and infection can often go undetected. The Intradermal Tuberculin test is considered the standard tool for the diagnosis of tuberculosis. However, it is often questioned due to false positive or negative results (Bonovska et al., 2005; Gong et al., 2017). The possible alteration in the response to the Tuberculin test as a result of mishandling has been ruled out in this research because the reagent used, has already been tested in cattle. However, in this study it is suggested to reevaluate the cases of primates that registered reactions using serological, radiographic, pathological, microbiological and cytological tests, due to the high risk of transmission of the disease, which could easily spread in relation to the origin of these animals, management of their habitat, feeding and proximity to humans and other species within the wildlife centers.

## **Conclusiones**

In the present study, 0% of positive cases of tuberculosis were detected in the population of non-human primates in captivity in the two wildlife centers of the city of Guayaquil, of the species *Saguinus fuscicollis*, *Cebus aequatorialis*, *Sapajus apella*, *Saimiri cassiquiarensis*, *Alouatta Palliata* and *Ateles geoffroyi*, by intradermal test with inoculation of Bovine PPD Tuberculin. However, there was a grade 1 postinoculation reaction in two primates of the study during the first 24 and 48 hours, considering this reaction as negative to tuberculosis, attributing the reaction to contact with infected humans or animals, basing this criterion on the results obtained. In addition, a p value of 1 ( $> 0.05$ ) was obtained, so it is concluded that there is no difference between species with respect to the degree of reaction, however, there is a difference in the number of reactions with the primates in the study. The exposed

results contribute significantly to the monitoring of wildlife animals, visualizing a current panorama of non-human primates in the country and mainly contribute significantly to the control of tuberculosis, a threatening disease for humans and animal species.

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