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Unveiling the Threat: Case Reports of Extra-Pulmonary Tuberculosis among Sanctuary Chimpanzees

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Abstract

Primate sanctuaries across Africa play a pivotal role in the rescue and rehabilitation of confiscated and rescued wild primates, many of whom have had extensive contact with humans prior to their arrival and throughout the rehabilitation process, heightening the risk of disease transmission. While tuberculosis is not naturally occurring in free-living chimpanzees, it has been extensively observed in captive primates that have been in close proximity to humans or other captive primates infected with *Mycobacterium tuberculosis*. This case report delves into an outbreak of extra-pulmonary tuberculosis among juvenile chimpanzees within a sanctuary, detailing the associated diagnostic challenges and treatment approaches. The five cases had close contact with a caregiver infected with tuberculosis, subsequently transmitting the infection to other in-contact chimpanzees. Prolonged treatment, employing the human protocol of quadri-therapy (rifampicin, isoniazid, pyrazinamide, and ethambutol), followed by bi-therapy (rifampicin and isoniazid), resulted in complete resolution for all five cases. These cases underscore the critical importance of maintaining high levels of biosecurity, implementing effective quarantine measures, and adhering to strict hygiene practices when working with non-human primates.

Keywords

Extrapulmonary Tuberculosis, Tuberculosis, Chimpanzees, Zoonotic Risk, Biosecurity, Diagnosis Challenges

1. Introduction

Across the globe, wild primates face a multifaceted array of threats including but not limited to poaching for bushmeat consumption and illegal trade, habitat degradation through encroachment, and the omnipresent spectrum of diseases [1]. In this context, African primate sanctuaries emerge as pivotal bastions, serving a crucial role in the comprehensive rescue and rehabilitation of seized and rescued wild primates, addressing both their physical and psychological needs. At the forefront of this conservation effort stands the Pan African Sanctuary Alliance (PASA), a preeminent coalition of wildlife centers across the African continent [2]. PASA members stand resolute in their commitment to safeguarding the future of Africa's primate population, embarking on a multifaceted mission that encompasses combating illicit wildlife trade, orchestrating the recovery and rehabilitation of trade-affected orphans, ensuring the well-being of wild primate populations and their natural habitats, and fostering knowledge dissemination and community empowerment [1]. The bedrock for superior care standards for sanctuary primates is provided by the meticulously crafted PASA operations manual and veterinary compendium [2].

The journey of confiscated and rescued primates frequently intersects with human proximity, both prior to their arrival at rehabilitation centres and throughout the rehabilitation process. These close interactions significantly heighten the latent risk of disease transmission, encompassing zoonoses and anthropo-zoonoses. The pervasive threat of zoonotic diseases, particularly tuberculosis (TB), poses a formidable challenge in primate sanctuaries, impacting both human and non-human primates. To address this challenge, PASA sanctuaries have implemented rigorous protocols, including stringent quarantine measures and comprehensive disease screenings for newly arrived primates. A minimum 3-month quarantine period involves initial triage, emergency treatment as necessary, and thorough health assessments with serial testing for common parasitic, bacterial, and viral diseases, including zoonotic diseases (**Supplementary Table S1** [3]). Only after this meticulous evaluation are new arrivals allowed contact or integration with resident chimpanzees. The health care regimen for these primates focuses on mitigating zoonotic hazards, particularly those arising from interactions between humans and great apes or monkeys [2].

2. Great Apes' Zoonotic Tuberculosis

In the context of captive primates, TB is prevalent, a stark contrast to its absence in their free-living counterparts [4]. This suggests that human-wildlife proximity plays a pivotal role in the transmission of *Mycobacterium tuberculosis* (MTB, or Koch's bacillus) to non-human primates [5]. On the contrary, while the zoonotic potential of non-human primates to transmit the disease to humans is recognized, actual instances of such transmission are rare [4]. The

transmission of MTB primarily occurs through respiratory tract aerosols produced by sick and contagious individuals, but alternative routes, such as the digestive and cutaneous tracts through mucous membranes or genitourinary injuries, are also possible [6] [7]. The infectious process and resulting lesions vary depending on the entry route of bacilli, as well as the virulence of the mycobacteria and the susceptibility of the host [6]. Horizontal transmission unfolds either through direct close contact or indirectly via shared environments, equipment, water, and food [7].

Primary lesions emerge as lung lesions, facilitating the excretion of bacilli [6]. Progressing through lymphatic pathways, these lesions reach satellite lymph nodes (LN), manifesting as latent and mostly inconspicuous primary TB infections that typically undergo spontaneous healing [7]. The trajectory of infection depends on macrophage control over bacterial growth, with the potential for regression, latency, or progression, particularly in cases of high infecting doses, youth, or immunocompromised states, leading to the development of TB disease [7]. Phagocytes can disseminate MTB via lymphatic pathways, spreading them to regional LN and throughout the body via lymph or blood routes [7], resulting in the formation of secondary lesions.

TB manifests in two primary clinical forms: pulmonary TB (PTB) and extrapulmonary TB (EPTB). While PTB is the predominant clinical form in humans, EPTB encompasses TB affecting organs beyond the lungs, such as LN, pleura, skin, abdomen, gastrointestinal tract, genitourinary tract, joints, bones, and meninges [8]. Among these, TB lymphadenitis stands out as the prevailing type of EPTB in both humans and non-human primates, often presenting as cervical adenopathy. However, inguinal, axillary, mesenteric, mediastinal, and intramammary cases have also been documented [9]. Peripheral LN lesions typically appear as "yellowish-white to gray caseous granulomas", varying in size from small, sub-millimeter lesions to larger coalescing formations [7]. Notably, the process of lesional calcification is less frequent in non-human primates compared to humans and bovids [6].

3. Tuberculosis Diagnosis Procedures

In humans, the majority of TB cases stem from primary TB following initial infection. However, latent TB infection represents a significant portion of active TB, wherein the bacteria can remain dormant for decades without inducing clinical signs. The risk of transitioning from latent to active disease amplifies when individuals concurrently experience pathologies that compromise their immune systems [10]. Similarly, primates below the age of 5 face a heightened risk of progressing from latent TB infection to active TB. Therefore, TB screening constitutes a crucial aspect of annual health assessments for both human and non-human primates in African sanctuaries. Various diagnostic tests, often employed in combination, are used to evaluate the health status of captive chimpanzees. These tests are from three categories: (a) Immunological diagnostic

tests that target the host's immune response; (b) Bacteriological diagnostic tests designed to detect the presence of Mycobacteria (cultures, stained smears); (c) Molecular essays designed to detect the presence of the *M. tuberculosis* DNA (**Figure 1**).

The immunological diagnosis serves the purpose of elucidating the host immune response, providing insights into whether an individual has been exposed to MTB. It plays a crucial role in the clinical follow-up of known infected chimpanzees and constitutes an indispensable tool for assessing the TB status and its evolution within the captive chimpanzee population [6]. The intradermal tuberculin skin test (ITST) or intradermal reaction (IDR) stands as the reference screening test for TB in mammals. This *in vivo* analysis of the cell-mediated immune response to TB antigens induces a type IV hypersensitivity reaction through the injection of tuberculin. The resulting local inflammatory reaction induced by the release of lymphokines, typically evaluated 72 hours post-injection in non-human primates [7] (**Supplementary Table S2** [6] [11] [12]), is facilitated by the administration under general anaesthesia of 0.1 ml of bovine tuberculin intradermally at the upper eyelid (left by convention). Simultaneously, a comparative intradermotuberculination involving 0.1 mL of avian tuberculin is injected into the second eyelid (**Figure 1(a)**). The preference for a comparative test arises from the heightened reaction with avian tuberculin in the presence of infections by atypical mycobacteria, distinguishing it from bovine tuberculin [13]. This is particularly relevant in primates' sanctuaries where the reliability of a single test may be compromised. A positive tuberculin test necessitates epidemiological context consideration and supplementation with another diagnostic tool, especially in sanctuary settings as this test may have cross reactions with other types of mycobacteria and thus, turning into a less sensitive technique than the Interferon reaction [14]. In comparison with necropsy findings, the ITST demonstrates a sensitivity and specificity of 84% and 87%, respectively [14].

(a) (b)

Figure 1. Immunological diagnostic for TB complemented with nucleic acid amplification test (NAAT) (a) Second injection of avian tuberculin on the right eyelid of a chimpanzee for immunological diagnostic using comparative intradermal tuberculin skin test (ITST). (b) PCR method (GeneXpert®) in Africa for the molecular detection of MTB DNA and rifampicin resistance testing. ©Emeline Chanove.

An additional immunological test employed for TB screening is the interferon gamma release assay (IGRA), specifically the QuantiFERON®-TB Gold Plus (QFT®-plus, Qiagen, Venlo, Netherlands). This cell-mediated immune response blood test measures the immune reactions elicited by peptide antigens—ESAT-6 and CFP-10—which stimulate mycobacterial proteins. Notably absent in the BCG vaccine and non-tuberculous mycobacteria, these antigens serve as markers for TB exposure [11] [15]. The primary cellular immune response triggered by this assay results in the release of interferon gamma (INF-γ), a type 2 interferon produced by activated T lymphocytes in response to specific antigens. CD4 T lymphocyte populations contribute to immunological control through INF secretion, but this cytokine also activates macrophages to exert detrimental effects on mycobacterial growth and infected cells. INF, produced rapidly and species-specific, serves as an early marker for TB infection, detecting both active and latent forms of the disease [6]. This assay demonstrates an unmatched sensitivity (98.9%) and specificity (98.1%) [16].

Immunological diagnostic tests should always be complemented with bacteriological diagnostic tests and/or molecular assays (if available) as they constitute the ultimate definitive means of diagnosing TB [7]. Bacteriological diagnostic procedures involve the microscopic examination of stained smears from pathological products (such as mucus, pus, or feces), a process feasible on-site in sanctuary settings. Mycobacteria, being acid-fast bacilli, exhibit positive staining through the Ziehl-Neelsen (ZN) method, with reported sensitivity and specificity of 70% and 97.1%, respectively [17]. However, this method lacks the capability to differentiate MTB from other acid-fast bacilli and requires a high bacillary content (5000 - 10,000 CFU/mL) in the sputum for detection [12] [18].

Molecular detection of *M. tuberculosis* in biological samples such as biopsies, gastric or bronchoalveolar lavages, is commonly used in the clinical laboratory and is conducted by nucleic acid amplification tests (NAAT) such as PCR, and when possible, genome sequencing techniques. In Africa, the commonly used molecular diagnostic method for confirming clinical and immunological suspicions is the GeneXpert® (**Figure 1(b)**; Cepheid; Sunnyvale, CA, USA). This system integrates nucleic acid extraction and semi-nested real-time PCR analyses, providing a rapid and highly sensitive detection of the MTB complex together with a rifampicin (RIF) sensitivity test [19] [20]. Thus, it functions both as a genomic amplification test for swift TB diagnosis and as a rapid test for antibiotic sensitivity, particularly rifampicin resistance—a crucial marker for predicting multidrug resistance in TB [19] [20] [21]. The MTB assay and MTB Plus assay demonstrate high sensitivity ranging from 83% to 89% and high specificity ranging from 98% to 99% [12].

Culture is considered as a reliable method for microbiological diagnosis in active TB but not for latent TB [22] [23] [24]. However, it is not very practical for active clinical cases as the conventional culture method takes 2 to 8 weeks to yield an initial result delaying the final diagnosis and potential treatment [12]

[24] [25], and its manipulation, requires specific laboratory safety measures and well-trained technicians [24]. Still, sputum culture to confirm the presence of MTB is a highly sensitive diagnostic method 94% with a high specificity of 99% and remains a useful technique for phenotypic drug susceptibility testing (pDST). [12]

Other complementary diagnostic test such as pulmonary radiography can be used for chimpanzees to detect the pulmonary form of TB, but this test detects any pathognomonic changes but cannot be used alone to confirm a TB diagnosis. Since there is no gold standard for the diagnosis of latent tuberculosis infection and culture is a slow diagnostic method for the active form, TB diagnosis routinely relies on a combination of measurement of host immune responses to MTB antigens and detection of the organism using molecular techniques along with microscopy and/or culture [22] [23] [24].

4. Tuberculosis Treatment Protocols

Chimpanzees are endangered and protected species, and once TB is diagnosed, a specific treatment must be applied following the recommendations of specialist veterinarians from the PASA network as well as the guidelines from human TB specialists in the country. An array of antibiotics to treat TB have been available for over 50 years, however, one of the major obstacles to successful therapy is that TB treatment requires long-term multidrug combinations and strict adherence to treatment [10]. Eradication of TB once detected is very challenging in primates and treatment should be based on antibiotic sensitivity.

Globally, the current first-line chemotherapy for drug-susceptible TB involves a quadri-therapy based on rifampine (RIF), isoniazid (H), pyrazinamide (Z), and ethambutol (E) for the first 2 months, followed by a bi-therapy based on RIF and H for the next 4 months [10]. There is an international consensus on the recommended dosage of those anti-tuberculosis drugs based on weight in both adults and children that can be applied easily on chimpanzees (**Table 1** [25] [26]; **Supplementary Table S3** & **Table S4** [25]). An initial intensive phase of two months is often based on the combined administration of RIF, H, Z, and E (quadri-therapy), except in children where tri-therapy is performed as E has proven toxicity in young individuals. The quadri-therapy in adult formulation is used for individuals above 20 kg while the tri-therapy in paediatric formulation is used for babies and juveniles below 20 kg of body weight. A second phase's treatment, called maintenance or continuation, for a period of four months, is usually based on bi-therapy using RIF and H, both in adult formulation (dosage per pill: rifampicin 150 mg and isoniazid 75 mg) or in paediatric formulation (dosage per pill: rifampicin 75 mg and isoniazid 50 mg). TB can be cured over this 6-month period only if the organism is drug susceptible and therapy is completed with a strict daily drug regimen. However, if the organism is resistant to some of the drugs or if the patient is inconsistent with the therapy, then

Table 1. Optimal doses for first-line anti-tuberculosis drugs in adults and children applied on chimpanzees. Drugs most often combined and applied in Africa. Extreme dosage values are given between brackets. The combined action of each drug is based on the development of a therapy spread over six months according to the combinations {RHZE}, {RH}, or {RHE} and to the possible adverse effects [25] [26].

Drug	Adults	Children	Common combinations			Possible adverse effects
	Daily dose (mg/kg)	Daily dose (mg/kg)	{RHZE} (mg)	{RH} (mg)	{RHE} (mg)	
Isoniazide (H)	5 (4 - 6)	10 (10 - 15)	75	75	75	Peripheral neuropathy, liver dysfunction, optic neuritis, toxic psychosis, general convulsions, and rarely symptomatic hepatitis anemia, lupus-like syndrome, or arthralgias Individuals at risk of peripheral neuropathy, should additionally receive pyridoxine, 10 mg daily.
Rifampicine (R)	10 (8 - 12)	15 (10 - 20)	150	150	150	Gastrointestinal reactions and pruritus with or without rash; but fever, influenza-like syndrome and thrombocytopenia can also appear.
Pyrazinamide (Z)	25 (20 - 30)	35 (30 - 40)	400	-	-	Gastrointestinal intolerance.
Ethambutol (E)	20 (15 - 25)	20 (15 - 25)	275	-	275	Used only in adults because it can cause optic neuritis leading to impairment of visual acuity and color vision, blindness, but also ocular toxicity.
Streptomycin (S)	15 (12 - 18)	15 (12 - 18)	-	-	-	Skin rash, cutaneous hypersensitivity reactions, vestibular and auditory dysfunction.

definitive cure can be delayed.

Checking the effectiveness of the treatment is done by microscopic examination (sputum smear) in humans. In the case of chimpanzees, the effectiveness of the treatment is tested after 3 months by GeneXpert method using gastric and tracheal washing and biopsies of the LN if hypertrophied. This system detects the presence of MTB and the sensitivity or resistance to the antibiotics of choice (RIF, H, Z, E) can be assessed. The ITST will be of less interest because there will be most likely false positive. It is also interesting to use interferon at the end of treatment to see if the values have decreased compared to the beginning of the treatment. The emergence of MTB strains resistant to H, RIF or any fluoroquinolone and at least one of the three second-line drugs create the main threat to global TB control [10] [22] [24]. In this case, TB treatments are more complicated, not standardized and require longer treatment regimens with second-line agents and adaptation according to the individual treated (human or non-human primate), but the new classes of drugs against TB show promising results. Concerning RIF resistant therapy, it's often based on a prolongation of the first line antibiotics.

In instances of treatment relapse, failure, or when patients have been previously treated, a "retreatment" regimen is promptly initiated, presenting two potential alternatives. The first involves a regimen closely resembling the initial treatment, primarily employing first-line medications, often supplemented

with S for the initial two months, and potentially including E during the subsequent phase [25]. Lasting approximately 8 months, this protocol lacks uniformity in its global application. The second option, upon suspicion of resistance, entails recommencing treatment after the standard 6 months, using a quadri-therapy for 3 to 4 months, followed by a bi-therapy phase for 2 months, resulting in a total 12-month therapy cycle. In the event of suspected or confirmed resistant strains, second-line drugs become essential (categorized into three groups as in **Supplementary Table S5** [25]). Regimens and dosages lack international standardization and need adaptation based on the case, lasting between 18 to 24 months.

5. Case Study Rationale

Even aware of those diagnosis and treatment procedures, managing TB in chimpanzees exposed to infected humans is a challenge. After successfully facing a wave of contagion, we believe in the attainability of a comprehensive resolution protocol through a stringent treatment regimen aligned with human protocols. Our study presents a series of fully resolved EPTB cases in five juvenile chimpanzees, achieved via rigorous adherence to human treatment protocols. These cases were diagnosed subsequent to a confirmed human TB instance, underscoring the critical role of robust biosecurity measures, quarantine procedures, and stringent hygiene protocols within sanctuaries. This report serves to emphasize the imperative for heightened vigilance and strict preventive practices in these environments.

6. Case Series

Background

No cases of diagnosed TB were reported at the sanctuary before the outbreak. This episode commenced in January 2019 and concluded in April 2020. The first case was diagnosed in January 2019, followed by the second case in February 2019, and the three next cases in March 2019. These five affected chimpanzees, all from the juveniles' group totaling 14 individuals, shared the same housing, routine, and caregivers. Their daily activities involved walks in the forest, sharing space, food, sleeping arrangements, and playful interactions. The same caregivers tended to all five chimps. Upon diagnosis, these five chimpanzees were isolated in a newly established quarantine housing, placed away from other chimpanzees, while the other nine individuals in the juveniles' group were also isolated, refraining from bush walks until the situation was fully resolved. One of the keepers was diagnosed with active PTB on May 2017, subsequently relieved from animal duties and granted sick leave. TB screenings performed on the sanctuary's chimpanzees in 2017 had shown negative results, until clinical signs appeared nearly two years later, coinciding with the outbreak (**Figure 2**).

Case 1: Lymph node tuberculosis spreading to digestive tuberculosis

Initial TB Suspicion (January 2017): The case began with a 7-year-old juvenile

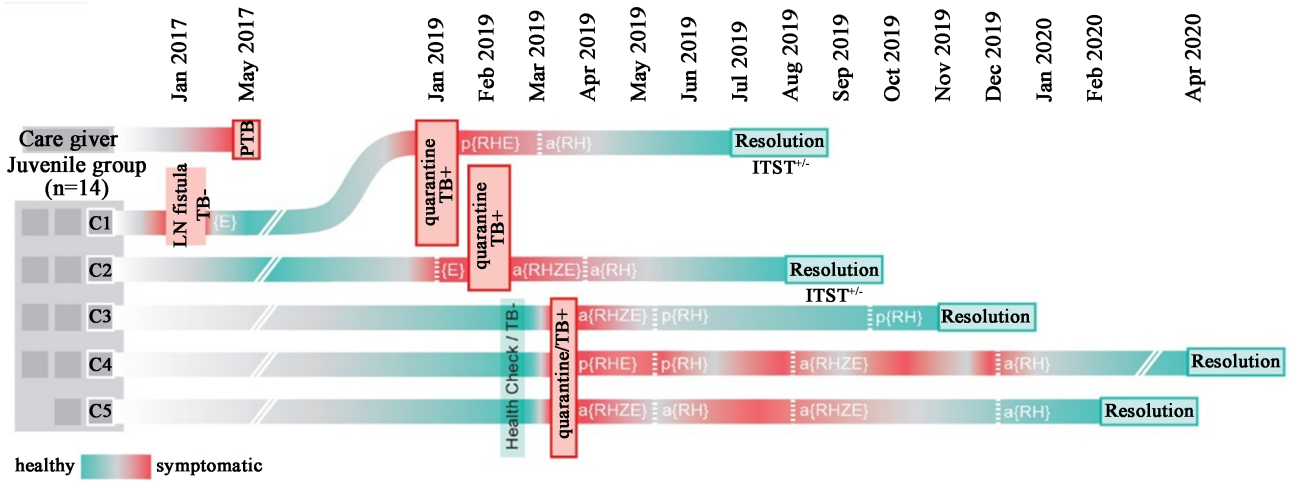


Figure 2. Timeline from TB outbreak to resolution of the 5 case reports ©Antoine Collomb-Clerc.

female chimpanzee (C1) displaying marked purulent mandibular and inguinal LN fistulas in January 2017. An ITST yielded a negative result. Given that no previous cases of TB had been reported in the sanctuary, the disease was initially dismissed after the negative result. A culture of the fistula's exudate identified Staphylococcus aureus, and treatment ensued based on antibiotic sensitivity, involving topical and oral erythromycin. This approach led to a gradual resolution of C1's symptoms. Three months later, one of C1's daily caregivers, engaged in various activities like forest walks, cleaning, feeding, playing, and general care, was diagnosed with PTB and successfully treated for six months. The caregiver was subsequently relieved from chimpanzee-related duties and declared cured at the treatment's conclusion.

TB Diagnosis (January 2019): However, in January 2019, two years after the resolution of initial clinical signs, C1 exhibited severe symptoms, including rapid weight loss (from 29 to 20 kg in 5 months), anorexia, nausea, intermittent diarrhoea, fluctuating body temperature, a grey skin color, and a reluctance to go outdoors with other chimpanzees. TB was suspected again, prompting a comprehensive diagnostic approach. Tests included a comparative ITST, gastric and bronchoalveolar washes (Figure 3), lung radiography, ZN staining on fecal samples, and general blood analyses. C1 was placed under strict quarantine, receiving symptomatic treatment while awaiting laboratory results. Three days later, only the ITST returned positive, with negative findings in lung radiographs, gastric and tracheal lavages on PCR for MTB, and ZN staining on feces. Hematology results showed anaemia and leucocytosis, while malaria, hepatitis B, and HIV tests were negative. Faecal culture was negative for *Salmonella* spp. As C1 continued to lose weight, repeat gastric and broncho-alveolar lavages five days later revealed a positive MTB result in gastric lavage PCR and RIF sensitivity.

TB Treatment: Following the advice of a local TB specialist, treatment commenced with the daily pediatric tri-therapy protocol. Within ten days, C1's general health improved, and her appetite returned. After two months of tri-therapy, she transitioned to the adult bi-therapy formulation. C1's rapid

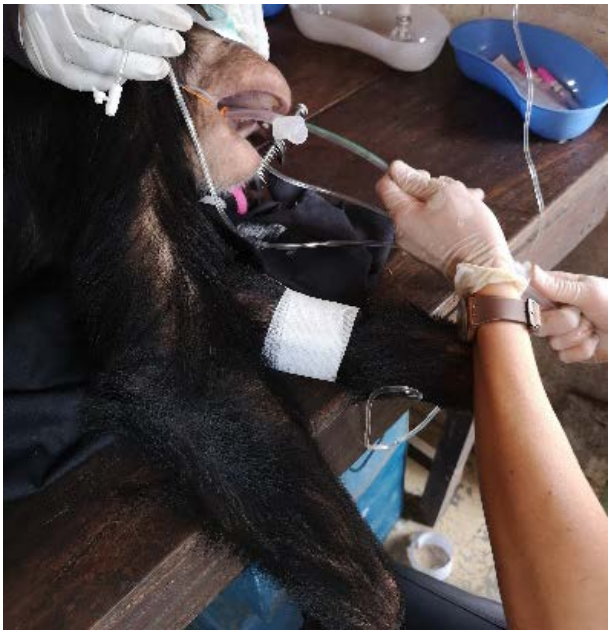


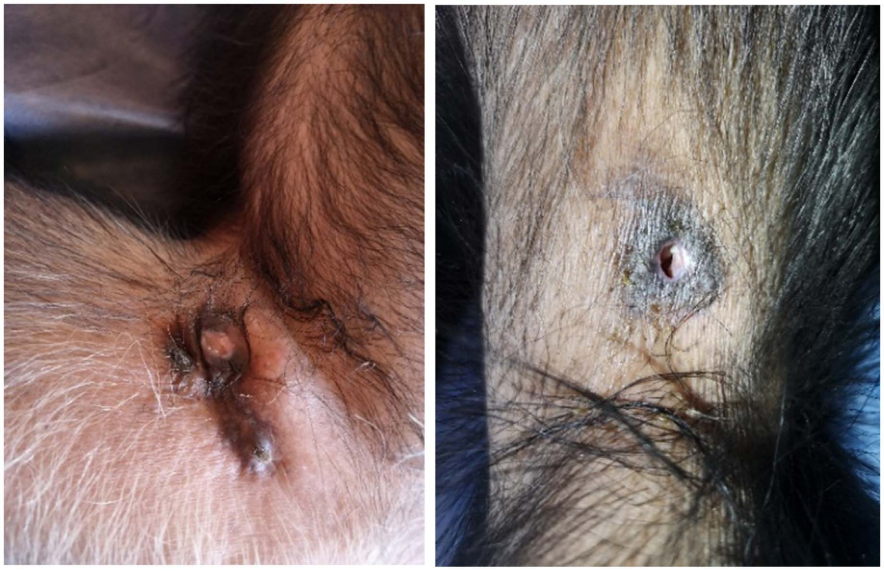
Figure 3. Gastric lavage on C1 ©Emeline Chanove.

improvement saw her weight increase to 30.1 kg by day 167 of treatment. After six months of treatment, diagnostic tests, including PCR on gastric and broncho-alveolar lavages, ITST, Interferon-γ ELISA test (Quantiferon®), and ZN staining on feces, were all negative for TB, except for a mild reaction in the ITST. Given the successful treatment response, drug therapy was halted, and strict quarantine was eased. Health checks twice a year, combined with daily visual monitoring, were implemented to monitor C1 and promptly identify any signs of disease recurrence.

Case 2*: Lymph nodes tuberculosis after contact with a positive case*

Initial TB Suspicion (January 2019): The second case (C2) involved a 6-year-old juvenile female chimpanzee, having direct contact with the previously TB diagnosed C1 for three years before exhibiting clinical signs. At a weight of 20 kg in January 2019, C2 presented severe lameness in the right leg, leading to an initial treatment with anti-inflammatory drugs (ibuprofen 100mg twice a day for 5 days). However, after 4 days on this regimen, lameness persisted, accompanied by hyperthermia (38.8˚C) and hypertrophy of the popliteal and inguinal LN. Two days later, purulent fistulas developed in these LN, mirroring the presentation observed in C1 in 2017 (**Figure 4(a)**, **Figure 4(b)**). Bacterial culture revealed multidrug-resistant *Staphylococcus aureus*. Differential diagnostics considered *Corynebacterium* spp., *Sporothrix* spp., *Mycoplasma* spp., or *Mycobacterium* spp. Antibiotic treatment with erythromycin and fluconazole showed no improvement after a month, and signs of clinical deterioration emerged, including extreme fatigue, undulating hypothermia in the morning and hyperthermia in the evening, and marked enlargement of the LN with necrosis.

TB Diagnosis (February 2019): TB became suspected, prompting C2's quarantine with stringent biosecurity measures. All ongoing treatments were



(a) (b)

Figure 4. Clinical signs on C2. (a) Hypertrophy and fistula on inguinal LN from C2. (b) Fistula on popliteal LN from C2 ©Emeline Chanove.

halted, and diagnostic tests included biopsies of the inguinal and popliteal LN, as well as gastric and bronchoalveolar lavages. GeneXpert® PCR results were positive for MTB and RIF-sensitive in LN biopsies but negative in both gastric and broncho-alveolar lavages.

TB Treatment: Quadri-therapy in an adult formulation commenced, yielding remarkable clinical improvements within five days. The LN's induration and enlargement significantly decreased, and C2's general state and demeanor improved, demonstrating a good appetite and heightened activity. After 18 days, the LN fistulas were closed and clean. Two months into treatment, bi-therapy in an adult formulation began, and by this point, no clinical signs were evident, with C2 weighing 25.3 kg. At the three-month health check, GeneXpert® results on bronchoalveolar and gastric lavages were negative, but the ITST remained positive. After six months of treatment, further health checks showed negative results for gastric and broncho-alveolar lavages, ZN staining on feces, and interferon tests. However, as observed in C1, the ITST showed suspicious results. Despite the chimpanzee appearing healthy with no clinical signs, and a weight increase to 28 kg, regular controls were implemented, and the individual was maintained in quarantine.

Case 3*: Lymph nodes tuberculosis after contact with two positive cases*

Initial TB Suspicion (March 2019): The third case (C3) involves a 5-year-old juvenile female chimpanzee, in direct contact with previously diagnosed cases C1 and C2. The same caregiver, previously diagnosed with PTB in 2017, had been caring for C3 during that period. No clinical signs were observed in C3 before a routine health check, prompting a swift implementation of thorough TB screening for any chimpanzee in direct contact with C1 and/or C2.

TB Diagnosis (March 2019): A battery of diagnostic tests was conducted, in-

cluding a comparative ITST, gastric and broncho-alveolar lavages, and ZN staining on feces and sputum/tracheal mucus. The ITST, after 3 days, displayed a positive grade 5 reaction for bovine tuberculin on the left eyelid, resulting in complete closure (Figure 5) and necrosis within a few days. Despite negative GeneXpert results from both lavages at this point, indicating no active TB, the chimpanzee's general state changed dramatically two weeks later, exhibiting lethargy and hypertrophy of the left mandibular LN. Isolation, strict biosecurity measures, and health control were immediately implemented. Three weeks post-ITST, the central part of the left mandibular LN started necrotizing and opened, revealing a whitish caseous mass (Figure 6). Fine needle aspirate and punch biopsies of the LN, along with gastric and broncho-alveolar lavages, ZN

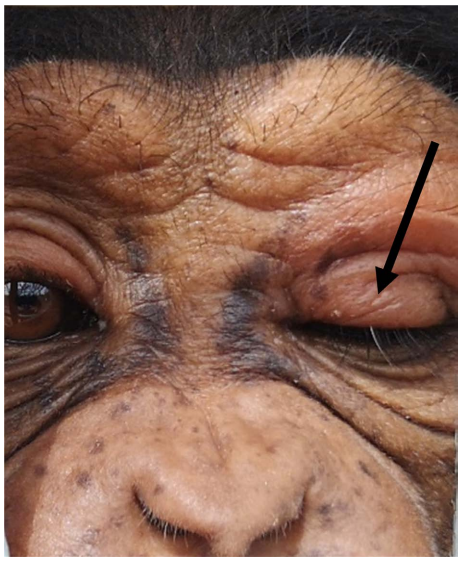


Figure 5. Result of ITST on C3: grade 5 on bovine tuberculin (left eyelid) ©Emeline Chanove.

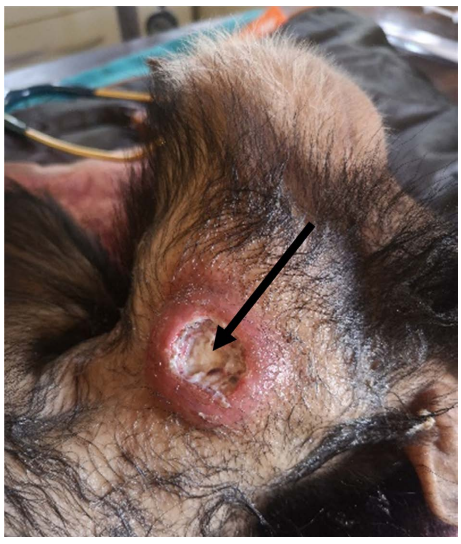


Figure 6. Clinical lesion at the mandibular LN of C3 (whitish caseous mass), three weeks after ITST ©Emeline Chanove.

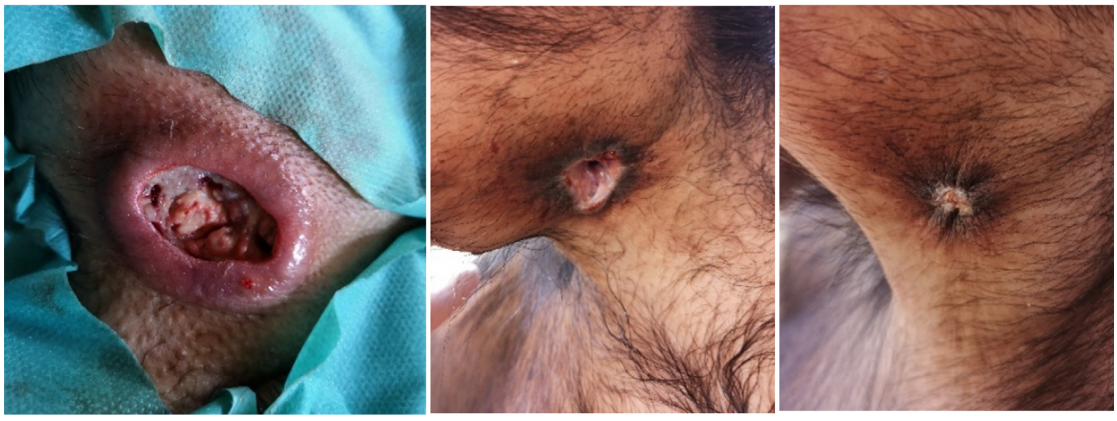
staining on feces and pus, and a Quantiferon® test, were conducted. Despite waiting for results, the juvenile chimpanzee's health continued to deteriorate, displaying lethargy, anorexia, weight loss, and cycles of hypothermia and hyperthermia similar to C1 and C2.

TB Treatment: GeneXpert results were positive for MTB and RIF sensitive in the biopsies of the hypertrophied and caseous mandibular LN but negative in gastric and broncho-alveolar lavages. ZN staining was negative on feces and pus, while the Quantiferon® test was positive with a high value (>0.34 UI/ml). Quadri-therapy commenced immediately after these results. Within two days of treatment initiation, C3 exhibited improvement, becoming more active and resuming eating. By day 5, the wound on the mandibular LN began closing, by day 16, the LN fistula was almost entirely closed, with only 0.3cm remaining, and by day 25 the wound was closed (Figure 7). After two months, C3 transitioned to the bi-therapy phase in pediatric formulation. At the six-month mark, a slight enlargement of the inguinal LN persisted. Following the TB specialist's advice, treatment was extended for an additional month. The last health check screening showed negative GeneXpert® results for all lavages and biopsies, although the interferon test remained positive, albeit with a lower value attributed to memory cells involved in the test. It was then decided to cease treatment and closely monitor the chimpanzee's health.

Case 4*: Lymph nodes tuberculosis after contact with two positive cases*

Initial TB Suspicion (March 2019): The fourth case (C4) involves a 5-year-old juvenile male chimpanzee in direct contact with previously diagnosed cases C1, C2, and C3. Additionally, C4 had direct contact during bush walks with the same caregiver infected with PTB in 2017. No clinical signs were observed in C4 before a routine health check, including an ITST.

TB Diagnosis (March 2019): Similar to C3, C4 underwent tuberculosis screening, including a comparative ITST, gastric and broncho-alveolar lavages, and ZN staining on feces and tracheal mucus. Three days post-ITST, a grade 5



(a) (b) (c)

Figure 7. Evolution of the mandibular LN of C3 during the TB treatment a. Aspect at beginning of the treatment (T0). b. Aspect at T0 + 11 days. c. Aspect at T0 + 25 days with complete closure. ©Emeline Chanove.

reaction with bovine tuberculin on the left side resulted in complete eye closure and subsequent necrosis after five days (Figure 8). Despite negative GeneXpert® results from lavages, indicating no active TB, and no other clinical signs present, C4, weighing 17.1 kg at this point, exhibited bilateral hypertrophy of the mandibular LN exactly one month after the intradermal test (Figure 9(a)). Biopsies of the lesions and lavages were conducted (Figure 9(b)), and C4 was placed in quarantine as a precaution. Biopsies confirmed MTB positivity but RIF resistance, while subsequent lavage retesting revealed MTB positivity and RIF sensitivity. ZN staining on pus, feces, and mucus yielded negative results.

TB Treatment: A standard tri-therapy on pediatric formulation commenced based on the chimpanzee's age and weight, as advised by the local TB specialist. After five days of treatment, LN hypertrophy began decreasing. However, after 12 days, the mandibular LN became indurated again, and a fistula appeared on day 22, with the chimpanzee maintaining the same weight. After one month of treatment, a second GeneXpert® test was conducted to reassess treatment sensitivity,

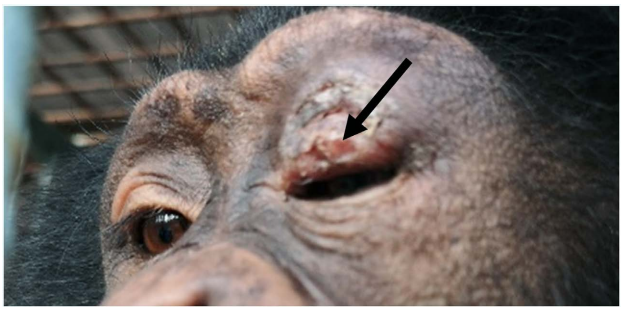


Figure 8. ITST result on C4 after 5 days: Grade 5 on bovine tuberculin (left eyelid) with necrosis ©Emeline Chanove.

(a) (b)

Figure 9. Clinical aspect of the lesions on C4 before any treatment (a) Bilateral mandibular LN hypertrophied on C4. (b) Biopsies done on clinical lesions on C4 ©Emeline Chanove.

confirming MTB positivity and RIF sensitivity in biopsies. The transition to the bi-therapy phase followed, similar to the previous case.

After 118 days of treatment, a left inguinal LN hypertrophy emerged, prompting the specialist's recommendation to continue treatment and revert to quadri-therapy for four months, suspecting resistance to at least one treatment molecule. At day 190 of treatment, positive results for the TB skin test and interferon test were reported, while all GeneXpert tests (biopsies and lavages) returned negative. Despite some persistent clinical signs on the LN, treatment extension was advised. After 265 days, the inguinal LN began to decrease and heal. By day 312 of treatment, the bi-therapy phase resumed for two months. The entire treatment concluded precisely after one year, during which all clinical signs disappeared, and C4's general state became healthy. At this point, the chimpanzee weighed 21.5 kg and exited quarantine.

Case 5*: Lymph nodes tuberculosis after contact with two positive cases*

Initial TB Suspicion (March 2019): The fifth case (C5) involves an 8-year-old juvenile male chimpanzee in direct contact with previously diagnosed cases C1, C2, C3, and C4. Before a routine health check and ITST, C5 displayed no clinical signs. Additionally, this chimpanzee had close contact with the initial TB-positive caregiver in 2017.

TB Diagnosis (March 2019): Similar to C3 and C4, C5 underwent a series of tests for TB screening, including a comparative ITST, gastric and broncho-alveolar lavages, and ZN staining on feces and tracheal mucus. Despite negative results for MTB in lavages, the ITST returned positive with a grade 4 reaction on the left eyelid (Figure 10). As no other tests indicated active TB, C5 was placed under observation, weighing 21.3 kg. One month after the initial ITST, C5 exhibited hypertrophy of the left mandibular LN, escalating from 2 to 10 cm in just 4 days (Figure 11). A biopsy of the lesion was sent for GeneXpert analysis, and within two days, the entire mandibular area became indurated (Figure 12). While awaiting results, clinical signs evolved, with the central area of the left mandibular LN developing a fistula and the chimpanzee showing

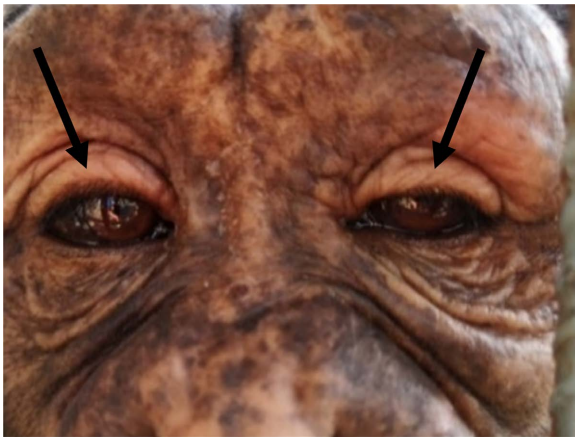


Figure 10. ITST result on C5: grade 4 on bovine tuberculin (left eyelid) with drooping of the eyelid and grade 3 on avian tuberculin (right eye) ©Emeline Chanove.

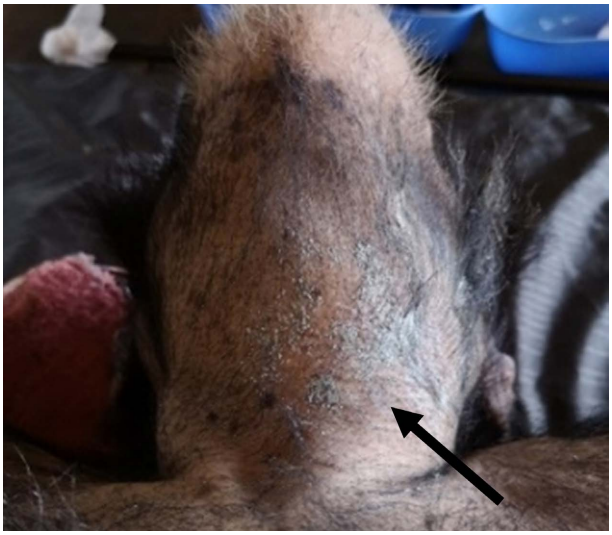


Figure 11. Hypertrophy of the left mandibular LN on C5 one month after the initial ITST ©Emeline Chanove.

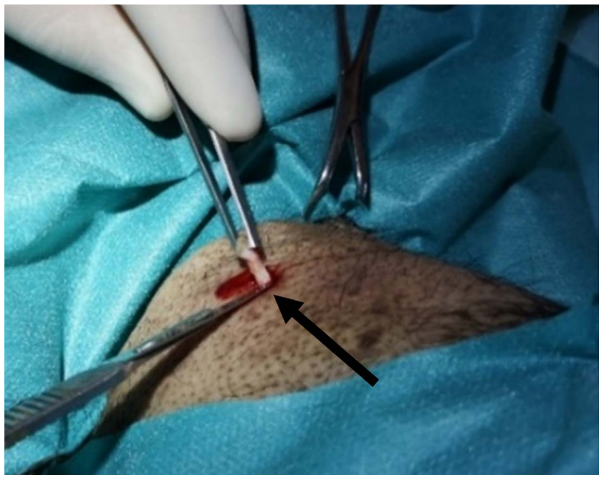


Figure 12. Biopsy on left mandibular LN on *C*5 ©Emeline Chanove.

digestive symptoms, including intermittent diarrhea. GeneXpert results from the biopsy were positive for MTB and RIF sensitive, but negative on gastric and broncho-alveolar lavages.

TB Treatment: Treatment commenced with quadri-therapy in adult formulation. Clinical signs resolved rapidly, and the LN size decreased from a 10 cm hypertrophy on day 1 of treatment to a 3 cm hypertrophy on day 6. At day 18, the LN fistula reopened with purulent discharge, but 14 days later, it closed completely, and the LN returned to normal size (**Figure 13**). After 61 days, bi-therapy in adult formulation began. However, by day 145, the left mandibular LN showed renewed hypertrophy (around 3 cm) and became indurated. Suspecting treatment resistance, on day 166, the specialist advised continuing with adult formulation quadri-therapy for 4 months, followed by a second bi-therapy phase for 2 months. The second phase of quadri-therapy began on day 218, leading to noticeable clinical improvement by day 308, with C5 weighing 41 kg.

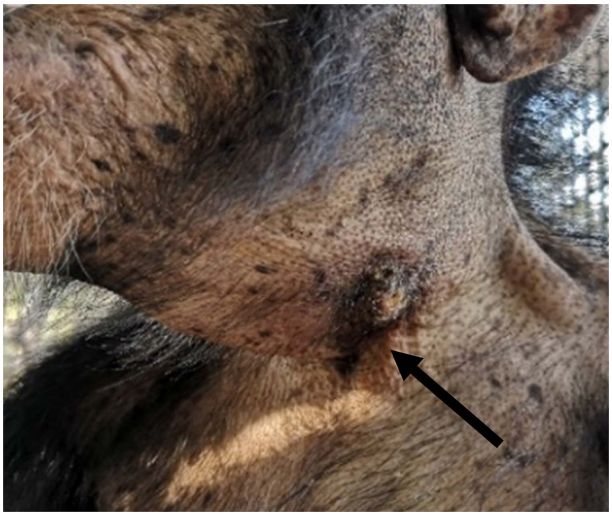


Figure 13. Improvement of left mandibular LN after 32 days of treatment on C5 ©Emeline Chanove.

After 312 days of treatment, no clinical signs remained, and the second bi-therapy phase commenced. By day 367, the chimpanzee was healthy, weighing 47 kg. The quarantine ended after the last negative GeneXpert® result at the end of treatment.

7. Discussion

TB primarily manifests as an infectious disease in humans, with limited documented instances of transmission to wild primates [27]. However, TB has been largely described in captive primates [28]. The potential human origin of the TB infection reported in this study involving TB lymphadenitis in orphaned juvenile chimpanzees is posited. The likely source of transmission is traced back to a caregiver diagnosed with PTB in March 2017, who maintained direct daily contact with the cohort of young chimpanzees in which all 5 cases of TB lymphadenitis were observed. This contextual background underscores a prominent health challenge faced by orphaned or rescued chimpanzees in African sanctuaries, attributable to the close physical proximity between primates and caretakers, particularly in the case of young chimpanzees [29].

Despite the delayed identification of chimpanzee C1 as TB positive in January 2019, it is postulated that the presence of a LN fistula, treated in January 2017, was indicative of an undiagnosed TB infection transmitted from the caregiver. Thus, this case series first highlights the fact that diagnosing TB can be very challenging in sanctuaries and cannot rely on one test only as false negatives and false positives can be present and hide latent diseases in captive population for years. Notably, chimpanzee C1 demonstrated a unique disease progression, characterized by the spontaneous resolution of LN fistulas followed by a latent period lasting two years, and subsequent re-emergence of the disease in a distinct extrapulmonary location, particularly within the digestive system. This case underscores the complexity of TB dynamics in non-human primates and warrants further in-

vestigation into potential reservoirs and transmission patterns. The five cases displayed diverse forms of extrapulmonary EPTB, also supporting a more varied disease expression compared to humans, who typically present with PTB.

8. Aetiology of TB Lymphadenitis, Transmission, and Biosecurity Rules

In the context of captive chimpanzees experiencing EPTB, the precise aetiology remains elusive; however, it is unequivocal that the implementation of stringent biosecurity protocols is imperative to curtail the potential transmission of infectious diseases in both directions [29].

The primary biosecurity protocol mandates a minimum 3-month quarantine period for any new chimpanzee entering the sanctuary, during which a comprehensive health assessment is conducted, encompassing screenings for infectious diseases such as TB, hepatitis, retroviruses, and faecal pathogens, as well as assessments of haematology and biochemistry. To clear individuals from quarantine and mitigate the risk of infection spread, three negative TB tests are mandated at arrival, mid-quarantine, and the conclusion of the 3-month period. Recognizing the limitations of relying on a single diagnostic test, a combination of tests is recommended, avoiding dependence on a singular negative IDR. The second biosecurity measure advocates for dedicated quarantine caregivers, whenever feasible, equipped with personal protective gear, including gloves, boots, a blouse with long sleeves or coverall, and a mask, to prevent potential contamination. The third rule underscores adherence to established hygienic protocols, such as rigorous hand washing, disinfection of dedicated contaminated equipment, and surfaces [30].

Regrettably, adherence to these biosecurity rules may encounter challenges in sanctuary settings, potentially resulting in the transmission of infectious diseases, including TB, from in-contact humans to chimpanzees and potentially to other wildlife in the event of release [31] [32]. These 5 cases of TB, likely emerging from a contaminated caregiver, further highlight why robust quarantine practices, coupled with regular health checks and rigorous hygienic measures, are imperative for maintaining the health status of sanctuaries and their residents, contributing significantly to the mitigation of zoonotic risks associated with interactions with great apes.

9. Clinical Symptoms of a TB Lymphadenitis in Chimpanzees

TB is characterized by a slow and progressive development, often spanning months or even years, with acute phases that can markedly hasten its course and exacerbate symptoms [6]. Following C1's recovery from submandibular and inguinal LN fistula, TB remained latent for two years, underscoring the potential for asymptomatic or latent forms that vary across species and TB strains, with the capability to affect various organs and tissues [6]. Non-human primates, particularly more susceptible species, exhibit a seemingly accelerated evolution of

TB compared to other animals [6]. The case of C1 also highlights that TB lymphadenitis, in a latent stage for years, can resurface and extend to the digestive system, giving rise to alimentary TB with rapid deterioration. Our case series also documents the cutaneous form of TB, manifested by the appearance of cutaneous abscesses, a phenomenon previously reported in non-human primates [33]. Behavioral changes, characteristic of TB infections, are observed in non-human primates during the active stage of the disease, including indifference to the environment and reduced vigor in routine physical activities [33].

TB lymphadenitis emerges as the most prevalent manifestation of EPTB, with cervical nodes being the primary site in humans and non-human primates, along with involvement of inguinal, mesenteric, and mediastinal nodes [34]. Representing approximately 35% of global EPTB cases in humans, cervical LN are the most common site, accounting for 60% to 90% of these cases [31]. While the prevalence of TB lymphadenitis in chimpanzees remains unknown, our case series aligns with the disease manifestation in humans, where cervical lymphadenitis is the predominant EPTB manifestation in young individuals. Cervical TB lymphadenitis may manifest as unilateral or multiple masses, often non-tender and slow-growing over weeks to months, with fistula formation observed in up to 10% of human cases [31], a feature frequently noted in our case series.

The progression of TB lymphadenitis involves the multiplication of bacilli provoking a delayed hypersensitivity reaction, leading to hyperemia, swelling, necrosis, and caseation in the center of the lymph node [31]. This progression was distinctly observed three weeks after the ITST in C3. Inflammation can lead to the swelling of multiple nodes, with adhesion to adjacent skin causing induration and purple discoloration, as evidenced in C5. The initial tubercle formation and lymphoid hyperplasia may advance to caseation and necrosis, resulting in cold abscess formation when caseous material liquefies, leading to a soft fluctuant node with overlying skin discoloration. Spontaneous drainage and sinus formation may ensue, as observed in C1 and C2, often accompanied by a secondary bacterial infection, primarily *Staphylococcus aureus*.

TB lymphadenitis in humans may initially lack systemic symptoms, as seen in C3, C4, and C5, or manifest with low-grade fever, weight loss, or fatigue, as observed in C1. Fluctuating fever (hypothermia-hyperthermia) in C1 could be attributed to malnutrition resulting from severe diarrhea and lethargy, common features associated with TB [34] [35]. The presentation of LN features may vary across different stages of TB lymphadenitis (**Supplementary Table S6** [35]). If left untreated, TB lymphadenitis in an immune-competent host may follow a prolonged and relapsing course, occasionally interrupted by transient LN enlargement, fluctuation, and/or sinus formation [34] [35] or, in some cases, remain latent for years, as exemplified by C1.

10. Challenges of the Diagnosis of EPTB in Chimpanzee

The diagnosis of TB in primates is inherently challenging due to the non-

pathognomonic nature of symptoms, the variability of the disease presentation, and the delayed manifestation of clinical signs post-infection. Clinical examination alone is insufficient for accurate TB diagnosis, necessitating the use of complementary diagnostic methods. However, diagnosing TB in primates, especially in wildlife sanctuaries, remains intricate. The diagnostic tests employed in this case series underscore the complexity of TB diagnosis, emphasizing the need for a combination of bacteriological laboratory tests, immunological techniques, molecular assays and organic exploration (**Table 2**). TB specialists recommend at least two positive tests, either in series or in parallel, to confirm a TB infection, as a single negative test cannot conclusively exclude the disease.

The ITST is considered the reference test among immunological tests, capable of detecting new cases or indicating a TB infection. However, careful interpretation is required due to factors such as varying reaction intensity, possible cross-reactions with non-tuberculous mycobacteria, and the subjectivity of reading results in wild animals. A comparative ITST is preferred to minimize the risk of false positives or negatives. While ITST remains a vital screening tool, the case of C1 illustrates the limitations of relying solely on a negative result, emphasizing the necessity for multiple diagnostic tests. The interferon test, more widely available, emerges as a reliable method for diagnosing active or latent TB, offering advantages such as minimally invasiveness, speed, and objectivity.

Compared to other screening tests like ITST, the interferon test is minimally invasive and quantifiable, making it particularly suitable for wildlife applications. Studies in experimentally infected non-human primates demonstrate its ability to detect a significant response within a shorter timeframe than ITST. Notably, the interferon test distinguishes recent infections from old ones and exhibits higher sensitivity than the tuberculin test, especially in cases of fulminant tuberculosis or terminal anergy [35] [36]. In our case series, the interferon test played a crucial role in the final diagnosis of several cases, proving valuable

Table 2. Summary of the tests used in a sanctuary for TB detection on chimpanzees ©Emeline Chanove.

Used tests for TB	Detection and aim	Pros vs Cons
Tuberculin skin test	Detection of delayed hypersensitivity to the tuberculin antigen (Ag), constitute a routine exam to detect active or latent TB	Cheap & easy but low sensitivity and specificity—not reliable if it's the only test and tricky to interpret
Thoracic radiography	Detection of the pathognomonic changes, able to detect latent TB	On site but low sensibility and only for pulmonary tuberculosis
PCR/GeneXpert®	Detection of the DNA of mycobacterium, able to detect active TB but also exposure to TB. It can confirm a case	Reliable if clinical signs present but expensive & only for active TB
ELISA/Quantiferon TB Gold Plus®	Detection of the antibody (Ab) present, able to detect latent or active TB	Able to detect latent form but frequent crossed reactions & expensive test
Microscopy—ZN staining	Detection of the bacillus, able to detect active TB	Easy, feasible on site but low sensitivity & specificity

for C3, C4, and C5 with a value of TB1-nil > 0.35 UI/ml and/or TB2-n > 0.35 UI/ml indicating a positive result and a *M. Tuberculosis* infection.

The bacteriological tests, including ZN staining from faeces or pus, are valuable for triggering further testing to confirm or rule out a TB diagnosis. However, ZN staining alone has limitations, as it cannot distinguish between TB and non-TB mycobacteria. Its diagnostic value lies in suspicion, requiring additional testing for confirmation. Within molecular tests, GeneXpert®, relying on genomic amplification techniques such as PCR is an efficient tool for establishing a definitive diagnosis of active tuberculosis. This test proved useful in confirming all five cases in this study.

Interestingly, C3, C4, and C5, despite showing no clinical signs before the ITST, developed lymphadenitis with fistulas and caseous discharge 3 to 4 weeks after the test. This raises questions about potential links between stress induced by health check exams, exposure to positive TB cases, and changes in environment leading to immune system suppression. The immunological test may have triggered a latent TB into an active stage, suggesting a need for further investigation with additional cases.

While these diagnostic methods are valuable, the discussion underscores the intricacies of TB diagnosis in primates, emphasizing the importance of a comprehensive approach utilizing multiple tests and careful interpretation to avoid false negatives and positives. The challenges highlighted in this study warrant ongoing research into refining diagnostic protocols for TB in non-human primates in sanctuary settings.

11. TB Treatment and Possible Resistance

The young chimpanzees who reacted positively to ITST were immediately isolated in quarantine with support therapy. Specific TB treatment only started after confirmation with GeneXpert® of MTB positive and RIF sensitive, following TB specialist advice to never treat an individual if diagnosis and sensitivity to the treatment have not been established. Each chimpanzee received treatment in the morning, on an empty stomach, according to their age, weight and to the phase of their treatment: quadri or tri-therapy for the first 2 months followed by bi-therapy for the next 4 months for each chimpanzee. [37] [38] Every day, the clinical condition of each chimpanzee was evaluated, and every three months, a complete TB screening was carried out to evaluate the evolution and the efficacy of the treatment. The ITST was positive after 3 months of treatment in all cases, but only suspicious after 6 months of treatment. This result is consistent and plausible as ITST detects the delayed hypersensitivity to the tuberculin Ag, detecting exposure or old/previous infection. The interferon values were lower but still positive after treatment, while the PCR using the GeneXpert® method was negative after treatment.

At the end of the initial 6-month treatment, 3 out the 5 chimpanzees show resolution of superficial lymphadenopathy and good clinical resolution. For C4

and C5, the mandibular LN for one and the inguinal LN for the other were still hypertrophied at the end of the six months treatment and the PCR was still positive for one of them. Therefore, resistance to treatment was likely for these two chimpanzees. On the advice of specialists, the treatment had to be continued for four months in quadri-therapy, followed by a second bi-therapy phase of two months. The total duration of specific treatment for these two chimpanzees was one year, after which no clinical signs were visible. The success of the TB treatment relies on the intensive monitoring and following up every individual case specifically and adapting the therapy if resistance is suspected.

12. Biosecurity Rules Necessary in Primates' Sanctuaries

In the 1980s, the term "biosecurity" emerged in the context of animal health and production systems, defined as the strategic planning and efforts aimed at safeguarding human, animal, and environmental health from biological threats. [39] Over time, the World Organisation for Animal Health (OIE) has refined the definition, now characterizing biosecurity as a collection of management and physical measures designed to mitigate the risk of introducing, establishing, and spreading animal diseases, infections, or infestations within an animal population. [3] In the realm of sanctuaries, the paramount objective of biosecurity is to shield against potential hazards posed by diseases and organisms.

Implementing biosecurity in primate sanctuaries involves key strategies such as exclusion, eradication, and control, facilitated by expert system management, practical protocols, and swift information exchange. The Pan African Sanctuary Alliance (PASA) provides guidelines specific to primate sanctuaries, emphasizing the importance of quarantine and maintaining a high level of biosecurity [30]. Member sanctuaries are mandated to adhere to regulations, including disease surveillance, strict hygiene protocols during quarantine, thorough cleaning and disinfection, maintenance of cleanliness standards in animal enclosures and processing areas, separation of captive and wild animals, and adherence to vaccination protocols. These measures collectively contribute to the overall biosecurity framework, ensuring the well-being and health of the primate populations in sanctuaries and potentially in wild populations. [40] [27]

While PASA sanctuaries are dedicated to preventing the introduction of new diseases to both the resident animals and the wild population, as well as minimizing the risk of staff infection, it is essential for every sanctuary to establish comprehensive contingency plans in the event of the failure of these preventive measures. Such plans must be prepared to effectively contain, treat, and eradicate any potential exotic diseases introduced to the sanctuary, as exemplified in this case series.

In the unfortunate occurrence of an outbreak, be it TB, Ebola, or other diseases, PASA member sanctuaries have well-defined intervention plans accompanied by protocols. These protocols serve to prevent the transmission of a specific disease between the sanctuary and the natural environment, or between a con-

fiscation site and the sanctuary. Additionally, there are protocols in place to prevent pathogen transmission during an epidemic, and specific plans detailing how the sanctuary would operate and care for animals in the event of a human pandemic affecting employees. The overarching focus of disease control lies in preventing the transmission of pathogens, with intervention planning serving as a foundational element.

Importantly, these contingency measures are designed to be easily revisable to adapt to evolving risks and ensure the continued effectiveness of the sanctuary's response strategies.

Inextricably tied to the One Health concept, biosecurity stands as a crucial element in curtailing the dissemination of diseases among humans, animals, plants, and the environment. Primate sanctuaries operate within environments where all facets are intricately interconnected. The implementation of a rigorous biosecurity level holds the potential to mitigate the repercussions of infectious diseases on public, animal, and plant health. Beyond these domains, it also extends its influence to the economy, the environment, and society at large. Recognizing the interdependence of these factors underscores the imperative of maintaining a stringent biosecurity framework, emphasizing its far-reaching impact on the holistic well-being of ecosystems and communities [3].

13. Conclusion

In conclusion, this case series sheds light on the occurrences and successful treatment of extrapulmonary lymph node tuberculosis in five young chimpanzees, emphasizing the profound implications of daily human-chimpanzee interactions within sanctuary settings. Despite the diligent implementation of biosecurity measures and quarantines, the unavoidable nature of these interactions exposes inherent risks. The diagnostic and treatment complexities associated with extra-pulmonary tuberculosis in primate populations underscore the critical need to incorporate tuberculosis screening as a fundamental component of routine health checks in primate sanctuaries, particularly in tuberculosis-endemic regions. Against the backdrop of the contemporary global landscape, characterized by the emergence of novel pathogens and the susceptibility of sanctuary-residing chimpanzees to diseases for which they lack immunity, the urgency for a global consensus to support sanctuaries in adhering to life-saving biosecurity protocols becomes evident. Such consensus-driven measures hold immense potential to safeguard countless lives in these vital sanctuary environments.

Informed Consent

Informed consent was obtained prior to publication of this report.

Ethical Approval

Ethical approval was obtained from the relevant authority.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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Supplementary Materials

Table S1. Extensive summary of the diseases to screen in chimpanzees. [IUCN]

Viral disease	Bacterial disease	Parasitic disease	Fungal disease	Non-infectious diseases
SIV/HIV	***Mycobacterium* spp.**	*Strongyloides* spp.	Candidiasis	Cardiovascular disease
STLV/HTLV	*Mycobacterium leprae*	Whipworm	Dermatophytosis	Reproductive disease
SRV	Pathogenic *E. coli*	Hookworm		
Foamy virus (FV)	*Salmonella* spp.	Pinworm		
Polio	*Shigella* spp.	Oesophagostomum spp.		
Measles	*Campylobacter* spp.	*Taenia* (tapeworm, *hymenolepis nana*)		
Varicella zoster	*Yersinia* spp.	*Entamoeba histolytica*		
Herpes simplex 1- 2	*Streptococcus pneumoniae*	*Balantidium coli*		
Epstein-barr virus/LCV1-2	*Klebsiella* spp.	*Giardia* spp.		
Cytomegalovirus (CMV)	*Brucella-like* organism	*Cryptosporidium* spp.		
Hepatitis A, B, C	*Treponema pallidum pertenue*	*Microfilariae*		
SARS-Cov-2	*Clostridium tetani*	*Trypanosima brucei*		
Adenovirus		Malaria		
hRSV		Sarcoptic mange		
Metapneumovirus				
Influenza				
Yellow Fever				
Ebola				
Rabies				

Table S2. The tuberculin intradermal reaction (IDR) scoring system in chimpanzees.

Grade	Description	Conclusion
0	No reaction	Negative
1	Ecchymosis, blood extravasation on the eyelid related to the injection	Negative
2	No swelling, variable degree of erythema	Negative
3	Varying degree of erythema with minimal swelling, slight swelling without erythema	Suspicious
4	Moderate swelling with drooping of the eyelid, variable degree of erythema	Positive
5	Severe swelling and/or necrosis of eyelid	Positive

It is advisable to perform an initial check of the injection site at the end of 24 hours then at 48 hours and 72 hours post-injection, looking for possible swelling and/or induration, the erythema being considered non-specific. IDR test is simple but require well-trained personnel for injection and result interpretation. False positives are also very frequent in case of high prevalence countries, vaccinated individuals (humans) and non-tuberculous mycobacteria cases.

Table S3. Formulation of tablets found in Africa.

		Rifampicine (in mg)	Isoniazide (in mg)	Pyrazinamide (in mg)	Ethambutol HCl (in mg)
Quadritherapy	Adult	150	75	400	275
	Pediatric	75	50	150	/
Bitherapy	Adult	150	75	/	/
	Pediatric	75	50	/	/

Table S4. Number of pills in TB treatment for adults according to the weight.

Months of treatment	Product	Weight in kg		
		30 - 39	40 - 55	>55
1 - 2 months of intensive phase	{RHZE}	2	3	4
3 - 6 months of continuation phase	{RH}	2	3	4

Table S5. Second line anti-tuberculosis drugs according to Gentilini and co mainly used in humans.

Bactericidal drugs	Aminosides (injectables only): Kanamycine (Km), Amikacine (Am), Capréomycine (Cm), Viomycine (Vm) Fluoroquinolones: Movifloxacine (Mfx), Levofloxacine (Lfx), Gatifloxacine (Gfx)
Bacteriostatic drugs	Ethionamide (Eto), Prothionamide (Pto), Cycloserine (Cs), Terizidone (Trd), Acide para-aminosalicyloque (PAS)
Drugs whose activity is still unclear	Clofazimine (Cfz), Linezolide (Lzd), Amoxicilline/Clavulanate (Amx/Cln), Thioacetazone (Thz), Imipenem/cilastatin (lpm/Cln), Clarithromycine (Clr), Isoniazide (H)

Table S6. Description of the different stages of tuberculous lymphadenitis according to Jones and Campbell description.

Stages	Clinical signs associated
Stage 1	Large, firm, mobile, discrete lymph node
Stage 2	Large, rubbery node fixed to surrounding tissue
Stage 3	Central softening due to abscess formation
Stage 4	Collar stud abscess formation
Stage 5	Sinus tract formation