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A Guidance for Diagnosis, Control Measures, and Surveillance of Leptospirosis in Non-Human Primates From a Veterinary Perspective in a One-Health Approach

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ABSTRACT

Leptospirosis is an emerging zoonotic disease caused by pathogenic *Leptospira* spp., affecting a wide range of domestic and wild mammals including nonhuman primates (NHPs). Despite rising incidence rates in both NHPs and humans, there is a lack of comprehensive resources addressing leptospirosis in NHPs from a veterinary perspective in a One Health approach. The close phylogenetic relationship between NHPs, humans and the similarities in disease progression may reveal valuable insights into the pathophysiology, diagnosis, treatment, control measures, and surveillance of leptospirosis. This review synthesizes existing literature on leptospirosis in NHPs, focusing on diagnosis, control measures, and surveillance. It offers insights into the prevalence among NHPs, environmental and civilization factors affecting *Leptospira* spp. dynamics, and recommendations for the diagnosis, management, and monitoring of leptospirosis. The findings highlight the need for coordinated research efforts and integrated surveillance systems to reduce leptospirosis risk in NHPs.

1 | Introduction

Leptospirosis is an emerging zoonotic disease caused by pathogenic *Leptospira* spp. Leptospires are present on all continents, except for Antarctica and it is estimated that leptospirosis results in approximately 1 million human cases and 60000 deaths annually [1]. Additionally, *Leptospira* sp. affects a wide range of animals, including nonhuman primates (NHPs) [2–4]. Natural *Leptospira* sp. infections have been documented in wild and captive NHPs, involving both Old World primates and New World primates [5–17]. Both outbreaks and individual cases are reported, often associated with mortality. Recently, an increase in seroprevalence has been observed across various NHP species in zoos to multiple *Leptospira* spp. [8]. This increase in seroprevalence could be linked to various factors, including climate change, increasing urbanization, and increased pest populations [18, 19]. Moreover, the capacity of *Leptospira* spp. to persist in the environment directly or through chronic carriers, such as the brown rat (*Rattus norvegicus*), emphasizes the importance of a One Health approach [20].

Remco A Nederlof and Jaco Bakker contributed equally to this work to be shared as last authors.

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Despite the increasing seroprevalence and the associated zoonotic risks, there remains a significant gap in the literature concerning leptospirosis in NHPs. Although the zoonotic disease risk seems to be low, the close phylogenetic relationship between NHPs and humans, as well as the similarities in disease progression, may reveal valuable insights into the pathophysiology of leptospirosis in both humans and NHPs. The use of NHP animal models has been indispensable in understanding the biology, transmission, host colonization, and pathogenesis of *Leptospira* spp. [21–24]. Due to their similarity to humans, leptospira infection studies in NHPs may contribute to the understanding of infection and pathogenesis of leptospirosis in humans and vice versa but also facilitate the translation of diagnostic and treatment protocols, ultimately contributing to the health of both animals and humans within a One Health framework.

Leptospira sp. belong to the family *Leptospiraceae*, order *Spirochaetales*. Leptospires are non-spore-forming, gram-negative, tightly coiled, spiral-shaped bacteria. They are obligate aerobes and motile, owing to the function of two periplasmic flagella or endoflagella. This motility is suggested to play a role in leptospiral pathophysiology and infectivity. *Leptospira* sp. can be categorized into pathogenic and saprophytic groups [25]. Serological classification allows the discrimination of > 300 serovars that can be clustered into 30 serogroups based on the expression of surface-exposed lipopolysaccharides [25, 26].

Recent reviews have focused on specific regions, species, or a combination of both, leaving a gap in comprehensive, updated resources that approach leptospirosis in NHPs from a veterinary One Health perspective. To address this gap, a search was conducted for publications in academic literature databases, such as PubMed, Scopus, and Web of Science, using subtopic-specific word combinations including “leptospirosis” and “nonhuman primates”, to identify potentially relevant publications. We then evaluated reports that we considered clinically relevant. This review synthesizes current knowledge on leptospirosis in NHPs, with emphasis on diagnosis, surveillance, and control measures within a One Health framework.

2 | Leptospirosis in NHPs

2.1 | Seroprevalence

Outbreaks of leptospirosis have been reported mostly in captive NHPs [6–10, 12, 13, 16, 17]. A recent meta-analysis provides information on the seroprevalence of leptospirosis in wild and captive NHPs globally [27]. The overall seroprevalence rate was reported to be 27.2% with serovar icterohaemorrhagiae being the most prevalent in both wild and captive NHPs. Other studies reported seroprevalences of 11.5%–28.3% in captive NHPs, and 36.6% in free-living NHPs, respectively [8, 28, 29].

2.2 | Pathogenesis

2.2.1 | Pathogenesis and Pathophysiology

The pathogenesis and pathophysiology of leptospirosis in NHPs is assumed to be analogous to that observed in humans [24, 30]. The spirochaetes enter the body via cuts or abrasions in the skin, or through mucous membranes of the eyes, nose or throat without causing local inflammation [30, 31]. To successfully establish an infection, leptospires bind to extracellular matrix components and host cells.

In humans, the incubation period is approximately 7–12 days, but the onset of the disease is variable and may range from 1 day to 4 weeks after exposure [32–34]. In the advanced stages of the disease, the septicemic phase, tissue damage occurs in multiple organ systems. Both direct damage to tissues by *Leptospira* sp. and immune-mediated mechanisms, cytokine storms, are responsible for tissue and organ damage, deranged tissue microcirculation and endothelial dysfunction. In humans, vascular damage, reduced blood flow and diffuse intravascular coagulation with resulting thrombosis may result in tissue hypoxia and cell death, resulting in kidney and liver cell damage, meningitis, myositis, pulmonary hemorrhage, and even death [35]. Anti-leptospiral antibodies become detectable at about the seventh day of illness. As a result, humans and non-rodent mammals that survive the septicemic phase of infection usually eliminate the leptospires from their bloodstream around the seventh to tenth day of illness. However, following this stage, some bacteria may persist in the convoluted tubules of the kidneys for extended periods of time. It is generally assumed that in humans shedding of leptospires in urine starts in the second week of illness and lasts 4–6 weeks, although rare cases of leptospiruria that lasted several months have been reported [32–34]. In humans, there is no documentation of permanent organ damage after recovery from an acute infection. Recovery of renal function may take several months, but most patients recover completely at least after one year [36]. Even with severe tissue damage, complete recovery is likely to occur.

2.3 | Natural Host and Clinical Features in NHPs

Natural *Leptospira* sp. infections are reported in various NHP species [5–7, 10–13, 15–17, 37]. The clinical signs of leptospirosis are non-pathognomonic and may mimic those of a range of other infections and diseases. Clinical signs reported in confirmed leptospirosis cases in NHPs are summarized in Table 1 [6, 7, 10, 12, 13, 16, 17].

In addition, an unusually high rate of abortion in squirrel monkeys was observed [12]. Although this suggests a role of *Leptospira* sp. in inducing abortions in NHPs, attempts to isolate *Leptospira* sp. from these cases were unsuccessful at the time. It is suggested that abortion induced by leptospiral infection results from placental damage secondary to circulatory disturbances in the dam, and/or infection in utero. Abortion resulting from leptospiral infection, described in domestic cattle constitutes one of the principal manifestations of disease in domestic cattle [38–40]. Although the causality of abortion in NHPs is not proven, the association with other animal species strengthens the hypothesis.

In humans and NHPs, clinical leptospirosis is associated with multiple organ dysfunction, and routine blood biochemistry and hematology may be performed to support the suspicion of leptospirosis in a clinical setting. Although a range of biochemical or hematological abnormalities may be present, these are often nonspecific [41–43]. Liver function tests often reveal elevated

TABLE 1 | Reported affected nonhuman primate species and observed clinical signs.

Species	Clinical signs	Reference
Macaca sylvanus	Anorexia, apathy, death within 24 h	[7]
Callithrix penicillata	Dyspnea, salivation, and frothing at the mouth, death	[17]
Cebus sp	Depression, malnutrition, icterus, and lymphadenopathy	[16]
Lagothrix lagothricha	Lethargy, vomiting and diarrhea [1]	[10]
Callithrix kuhlii	Depression, diffuse icterus, reduced body, diarrhea, lethargy and mild icteric serum	[6]
Cebus apella	Acute death	[13]
Saimiri sciureus	Subacute death, acute illness, jaundice, pyrexia, abortion and dehydration	[12]

liver enzymes and bilirubin, though the latter may be clinically evident as jaundice.

2.4 | Gross and Histopathological Findings

An extensive and detailed gross and histopathological overview in NHPs has been provided by Wilson et al. [7, 17]. The main gross findings were described to be generalized jaundice, multiorgan congestion, and multifocal hemorrhages. The most consistent histopathological lesions were observed in the kidneys, liver, and lungs, and consisted of renal tubular necrosis, nephritis and hepatocellular necrosis and pulmonary hemorrhage. The general pathological features closely resemble those reported in human cases.

3 | Transmission of Leptospirosis

3.1 | Leptospiral Reservoirs

Most mammals can get infected by pathogenic *Leptospira*. Yet, not all infected animals can be considered as reservoirs [44]. The main difference between reservoirs and accidental hosts is considered to be the renal colonization following systemic infection in reservoir animals and the absence of renal colonization in the incidental hosts [44].

Unlike most mammals, rodents, especially *Rattus norvegicus*, are resistant to leptospirosis despite chronic colonization of their kidneys. As a result, they can remain chronic carriers, shedding leptospires in urine for life, and thus contribute to bacterial transmission. In addition, rats excrete the highest quantity of *Leptospira* sp. per milliliter of urine of the investigated animal species [45, 46]. Rat urine is at or above 6.5 pH, while the ideal pH value for survival of leptospires is close to neutral (6.5–7.2) [47, 48]. As a result, rodents are considered primary reservoirs of *Leptospira* spp., with brown rat populations being implicated as key reservoirs for outbreaks around the world. Other rodents, such as capybara (*Hydrochoerus hydrochaeris*), beavers (*Castor fiber*, *Castor canadensis*), squirrels and mice, are wildlife species that may also carry leptospires, but their role as reservoirs warrants further investigation [3, 44, 49–51]. *Leptospira* sp. prevalence in rats is described to vary considerably based on geographic location, with some countries reporting 0% prevalence and others reporting prevalences > 80% [52–56].

Other mammals, including humans and NHPs are mainly accidental hosts and may serve as temporary carriers, shedding *Leptospira* in the environment for a limited time [3, 12, 24, 44]. Although natural shedding in urine is not established yet, after experimental infection, leptospires have been detected in the urine of only a few infected NHPs [13, 22, 31]. Nonetheless, the introduction of a carrier animal could result in the formation of an endemic area [30]. Nevertheless, NHPs may play a role in zoonotic cycles but their exact role is unclear [37].

3.2 | Direct Contact Transmission

Urine is the main route for the excretion of *Leptospira* sp. in animal hosts. Transmission occurs either directly, through contact with animal urine or indirectly via exposure to contaminated water or soil (Figure 1). While rodent-to-NHP transmission has previously been suggested, the possibility of intraspecies transmission among NHPs cannot be excluded [7, 12, 30, 50]. In NHPs, urine pH typically ranges from 6.0 to 8.5 while leptospires prefer 6.5–7.2 [57–59]. Further studies are required to clarify the possible roles of horizontal and vertical transmissions, including those involving urine, sexual contact and nursing and the survival time of leptospires in soil [16, 55, 60].

3.3 | Indirect Contact Transmission

Once excreted via animal urine, leptospires require moisture or a body of water to survive and remain infectious [61]. *Leptospira* sp. have been demonstrated to survive for weeks to months in the environment under favorable conditions. Such conditions include high humidity, a neutral pH (pH 6.5–7.2), and an optimal temperature around 28°C–30°C [61, 62]. Pathogenic *Leptospira* sp. are unable to replicate in the environment; however, they can aggregate and form biofilms, which enhances their ability to persist under adverse environmental conditions [63]. In soil, pathogenic *Leptospira* sp. are described to be able to survive at a humidity of < 20% and a pH < 6 [62]. They are described to survive for 130 days at 4°C, 236 days at 20°C, and 316 days at 30°C, independent of the environmental pH [64]. Consequently, exposure to stagnant water and contact with mud are important risk factors for leptospirosis [6, 65, 66]. In contrast, large bodies of water pose a significantly lower risk, likely due to the greater

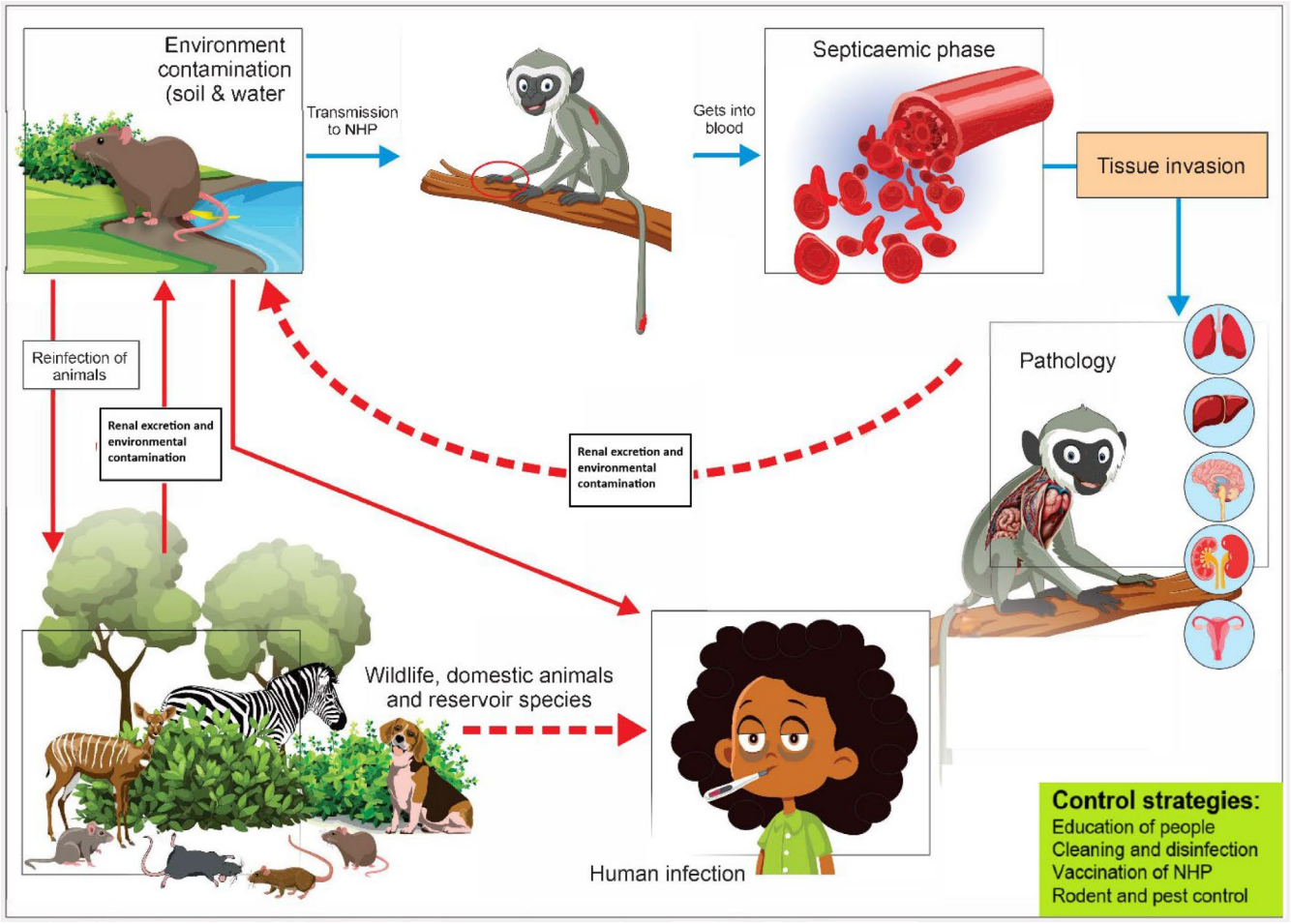

FIGURE 1 | Schematic diagram illustrating the mechanism of *Leptospira* infection in nonhuman primates, and the role of environmental contamination by reservoir species in facilitating transmission.

dilution of infectious urine [46, 65]. *Leptospira* sp. are sensitive to UV radiation, but biofilm formation and elevated water turbidity levels may shield bacteria from UV radiation [49].

With increasing evidence that *Leptospira* survive and persist in the environment, it has been acknowledged that field-based studies are overall underrepresented. Therefore, there is a need for assessments of the survival condition of *Leptospira* in natural environmental conditions to improve the understanding of the environmental stage of the leptospirosis transmission cycle [49].

4 | Diagnostics

4.1 | Diagnostic Considerations

The clinical manifestations of leptospirosis in NHPs are highly variable, with disease severity ranging from subclinical to fatal infection [6, 7, 10, 12, 13, 16, 17, 27]. Most infections, however, are subclinical and remain unrecognized in NHPs [27]. Therefore, the diagnosis of leptospirosis should rely on laboratory tests rather than solely on clinical signs.

Diagnosing the disease in NHPs is challenging, as all laboratory diagnostics are extrapolated from human medicine [6, 12, 43, 67]. The sensitivity of diagnostic approaches is highly dependent on the stage of the disease. In the acute phase, between Days 4 and 10 after the onset of clinical signs, bacteria are present in the bloodstream and cerebrospinal fluid. In the subsequent convalescent phase, which lasts between 4 and 30 days, *Leptospira* sp. are cleared from the blood, and IgM antibodies begin to appear. Analogous to human reports, if an NHP demonstrates clinical signs for more than 10 days, indirect diagnostic techniques may be preferred as well [68].

Diagnostic tests for leptospirosis are broadly categorized into those that provide direct evidence of infection—through the detection of leptospires or leptospiral DNA—and those that offer indirect evidence of exposure, such as the detection of anti-leptospiral antibodies [25, 43, 69–71] (Table 2). Samples should be tested at laboratories with appropriate biosecurity measures [69, 70, 72]. If available, a combination of samples, including both acute and convalescent serum samples, is recommended [43, 69, 70].

4.2 | Isolation and Identification of *Leptospira* sp.

Several diagnostic options for the detection of *Leptospira* spp. are available. The presence of viable leptospires in samples from infected NHPs may be confirmed by bacterial culture. The cultures can subsequently be used for serovar

TABLE 2 | *Leptospira* diagnostic options available after the onset of clinical disease that can be successfully used at a given time point. These are categorized into diagnostic tests that detect the presence of *Leptospira* spp. and tests that detect anti-leptospiral antibodies. The tests have proven effective in the areas shaded in clear green, while there remains uncertainty regarding their effectiveness in the banded green areas.

Time point (day)	1	2	3	4	5	6	7	8	9	10	11 +
Detection of *Leptospira sp.*				Blood culture							
				Cerebrospinal fluid culture							
											Urine culture
											Immunohistochemistry (renal tissue)
	Polymerase Chain Reaction (PCR)										
	Real-time polymerase chain reaction (RT-PCR)										
	Multilocus sequence typing (MLST)										
Detection of anti-leptospiral antibodies											Microscopic agglutination test (MAT)
								Enzyme-Linked Immunosorbent Assay (ELISA)			
								Loop-mediated Isothermal Amplification (LAMP)			

identification and for determining microbial sensitivity testing [72, 73]. Blood, cerebrospinal fluid and organ samples are useful within the septicemic phase of infection, which is approximately during the first 10 days after onset of clinical signs [43]. Urine samples should be cultured as soon as possible after collection, as *Leptospira* spp. are highly susceptible to unfavorable urine pH, which may rapidly reduce bacterial viability [43, 74]. In addition, *Leptospira* spp. exhibit slow growth, often requiring several weeks of incubation before successful isolation can be achieved [30, 43, 74]. The faster growth rate of saprophytic species may overgrow cultures and hinder the detection of pathogenic strains [74, 75]. For this reason, bacterial culture should be complemented by dark field microscopy (DFM), which allows for the identification of leptospires based on their characteristic morphology and motility [43, 72]. Once successfully cultured, *Leptospira* spp. can be further characterized using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS), a technique that has been shown to be a fast and reliable method for species-level identification [76–78].

It should be noted that DFM and culture have a low sensitivity and are expert-dependent for proper interpretation, and are therefore not recommended for routine diagnosis. Nevertheless, culture and serotyping may be valuable in prevention strategies [68, 72].

Direct light microscopic and phase contrast microscopic examination of urine samples may allow for the rapid detection of bacteria. To this end, leptospires present in samples can be concentrated using centrifugation. However, microscopic observation is labor intensive, requires experienced personnel and high biosafety levels, and has low diagnostic sensitivity, which may all contribute to high false-negative result rates [79, 80]. The specificity may also be adversely affected by the presence of fibrin and protein in the urine samples, which may be mistaken for *Leptospira* spp [43].

Immunohistochemical (IHC) techniques or silver impregnation staining methods offer high sensitivity and specificity by showing spirochetes within the renal tubules but require renal tissue samples [6, 17]. As a result, this technique is rarely useful in live animals but may assist in diagnosing leptospirosis postmortem.

4.3 | Nucleic Acid-Based Diagnosis of *Leptospira* spp.

Polymerase-chain reaction (PCR) constitutes an alternative technique for the direct detection of *Leptospira* spp. This technique can be utilized within the first week of infection to demonstrate the presence of leptospiral DNA in blood [81]. This test has a high sensitivity and specificity and may simultaneously allow for serotyping. In addition to classical PCR techniques, Real-Time PCR (RT-PCR) and multilocus sequence typing (MLST) have rapidly become commonly used diagnostic methods for leptospirosis [25, 43, 69, 71, 82, 83]. In humans, these molecular techniques are faster and more sensitive in the early stages of infection than the indirect diagnostic technique. Currently, MLST is a commonly used method for strain-level genetic typing of *Leptospira* spp. MLST can characterize isolates and allows for both the identification of species [84].

4.4 | Serologic Diagnosis

The microscopic agglutination test (MAT) is based on detecting agglutinating antibodies against *Leptospira* serovars using whole live *Leptospira* spp. The test involves mixing patient serum with live bacteria, incubating, and then observing agglutination by DFM [43, 69, 72].

The MAT allows for serogroup identification [4]. Veterinary diagnostic laboratories typically include 6–7 serovars, compared to > 30 different serovars used in human diagnostic laboratories [72]. In addition, it has been recommended that a panel should include both recent and locally circulating serovars [43, 70, 72]. This illustrates that assays that include many serovars are more sensitive yet more laborious and expensive to perform due to the maintenance of live *Leptospira* sp standard cultures [43, 69, 70, 72].

Sometimes, paired serum samples are needed to confirm the diagnosis by seroconversion or a rise in titer [69, 72]. In humans, a fourfold increase in antibody titer in the second sample, collected at least 10–14 days after the initial sample, is considered diagnostic for leptospirosis [43]. The MAT requires a sophisticated laboratory, precise timing of sample collection, and requires having

all the availability of circulating types of *Leptospira* serovars in live cultures [43, 69]. Although this test is highly specific, it has limited sensitivity in the acute phase, because anti-leptospiral antibodies are first detectable around 7–10 days after infection and peak within 3–4 weeks [70].

The MAT was the gold standard for the diagnosis of leptospirosis for decades. However, recent studies have shown that IgG and IgM-enzyme-linked immunosorbent assays (ELISAs) are more useful during subclinical infections and during the acute phase [69, 85, 86]. Unfortunately, the ELISA is not serogroup or serovar-specific [43]. Point-of-care serologic tests have been developed that detect *Leptospira*-specific antibodies in canine sera. Although these tests are useful for early diagnosis, caution should be applied as sensitivity and specificity may vary depending on circulating strains [72]. Therefore, point-of-care tests should be used together with recommended confirmatory tests for leptospirosis [87]. Nevertheless, a rapid test validated for NHPs would be beneficial to detect early disease. Currently, no application in NHPs is described in the literature, and additional research should be conducted to validate these tests in NHPs.

5 | Treatment

Leptospirosis should be treated as soon as possible, regardless of the severity of the disease or stage of the disease at the time of diagnosis. All therapies should be accompanied by appropriate supportive treatments, such as fluid therapy, anti-emetics, and nutritional support. Supportive treatments for the liver, e.g., choleretics and antioxidants, may be considered. Further supportive treatment should be guided by the severity and nature of clinical signs [88, 89].

Despite anecdotal attempts, no published reports confirm effective treatment of NHP leptospirosis [6, 12]. Trials investigating antibiotic treatments for leptospira-infected NHP are lacking. Empirical use of various antibiotics has been reported in two publications both unsuccessful. It remains unclear why the administered antibiotics were ineffective [6, 12]. Since infection is not uncommon in NHPs, we assume that treatment attempts are underreported.

In human samples, the presence of genes related to antibiotic resistance mechanisms in the *Leptospira* genus is described, but no similar investigations have been conducted in NHPs [90]. Moreover, in humans, the use of antibiotics in leptospirosis cases is a topic of debate, as their efficacy is not well established [91, 92]. Therefore, the empirical use of antibiotics in the absence of culture or PCR confirmation should be approached with caution.

Due to the lack of validated studies specific to NHPs there is no current therapeutic recommendation for NHPs. Although caution is warranted, until NHP-specific guidelines are available, extrapolated protocols from canine and human protocols may be the best option. Treatment protocols for dogs and humans include the administration of doxycycline at a dose of 5 mg/kg orally every 12 h for 14 days, which has been reported to eliminate the renal carrier state and may reduce the duration of clinical illness in humans and dogs. Oral use of amoxicillin (500 mg/day for 7–10 days), ampicillin (500–750 mg/day for 7–10 days), and azithromycin (500 mg/day for 3 days) have also been reported as treatment options in humans [89, 91, 93]. In severe human cases associated with renal and hepatic failure, the intravenous administration of penicillin G (penicillin G sodium; at a dose of 1.5 million U/6 h) is considered the treatment of choice [34].

6 | Control Measures

6.1 | An Integrative Plan: Generating Awareness

An integrative approach requires the active engagement of governments, caretakers, and veterinary professionals, with the promotion of awareness among the general public, representing a critical first step in reducing the incidence of leptospirosis [71, 94–96]. Targeted education of veterinary and animal care personnel regarding the zoonotic risks, transmission routes, and clinical manifestations of leptospirosis can substantially enhance the early detection and prevention of the disease. Well-informed staff are more likely to promptly report sightings of potential rodent vectors, strictly comply with hygiene protocols, and effectively contribute to the implementation of rodent control measures.

6.2 | Pest Control

An integrated pest management program is crucial in the prevention of leptospirosis [66, 97, 98]. Effective pest control should combine biological, mechanical, physical, and chemical measures, alongside ongoing surveillance [97, 99, 100]. Because of its complexity, rodent control management should be carried out promptly and professionally, as inadequate measures may lead to long-term and unpredictable consequences for NHPs and other non-target species, especially in zoological settings [101, 102].

Rodent control measures should involve the removal of wild vegetation and sealing off potential nesting sites and entrances to the NHP enclosures. Removal of leftover food at the end of the day, adjusting feeding frequency and total amount of offered food so that leftovers are prevented, is essential. Strict hygiene measures in storage facilities and warehouses are also necessary to reduce the local rodent populations [16, 17]. The guided disposal of household garbage, which is the major attractant of rodents to residential areas, should also be considered [17].

Mechanical methods may include a range of traps, although they are mainly used to reduce rodent population size rather than effectively eradicate rodent populations [103]. National regulations regarding the permitted use of (non-lethal) mechanical traps vary, as such traps are prohibited in Belgium and the Netherlands, (https://www.tweedekamer.nl/kamerstukken/kamervragen/detail?id=2025Z07223&did=2025D24176); nevertheless, in other parts of the world there are no explicit regulations governing the methods used for rodent control. It is therefore advised to check local regulations to avoid unnecessary fines.

Rodenticides are commonly formulated as baits in solid, liquid, or powder formulations, depending on the mechanism of

action and the biology of target rodents. However, their environmental persistence, potential for bioaccumulation, and associated risks to public and environmental health are inconsistent with regulations in many countries [103]. From a One Health perspective, it is essential to explore ecologically based rodent management strategies and implement sustainable alternatives to rodenticides [103–105].

6.3 | Housing Considerations for Captive NHPs

In addition to rigorous pest control practices, housing conditions of captive NHPs may be altered to reduce the risk of contact with potentially infectious rodent urine. Due to the risks of waterborne *Leptospira* spp., it is not advisable to use water from natural waterways as drinking water [106]. Additionally, small ponds may be present as enrichment features or may inadvertently form after rainfall. Water treatment processes will reduce the likelihood of leptospirosis in water ponds and moats [107]. Moreover, feeding stations and water bowls should be placed out of reach of rodents, and preferably drinking nipples should be used. Increasing the number of branches and elevated platforms for NHPs to rest on further reduces the risk of contact with rodent urine [16, 17].

Nowadays, habitat complexity e.g., enrichment, ponds, biomaterials as floor covering, is increasingly recognized as important for the maintenance of high levels of welfare for captive NHPs [108, 109]. However, this trend may conflict with traditional biosecurity measures designed to prevent leptospirosis transmission. Enclosures should be designed to facilitate regular cleaning and disinfection. Though hard, smooth, nonporous surfaces that can be sanitized are being gradually replaced in zoological facilities, they effectively prevent water accumulation and allow for easy and thorough disinfection [16]. Zoological institutions must aim to integrate both animal welfare and biosecurity considerations when designing enclosures and are encouraged to find alternative materials and enrichment features that allow for both.

6.4 | Cleaning and Disinfection Protocols

Under laboratory conditions, leptospires are slowly replicating organisms that are assumed to be sensitive to various disinfectants, high temperatures (> 42°C), hypertonic conditions, and acidic (pH < 6.0) or alkaline (pH > 8.5) environments [62, 110]. Routine cleaning and disinfection, starting with the removal of visible debris and organic material to improve disinfectant efficacy, are key components of hygiene protocols, and should equally apply to facilities housing NHPs [7, 111, 112]. These practices should be performed frequently including ponds or other water sources, as they may serve as environmentally contaminated reservoirs [113, 114]. Cleaning protocols should involve dry methods, such as fecal debris removal, and wet methods, such as high-pressure water cleaning, always followed by disinfection [111, 115]. Effective disinfection can be achieved either chemically, using appropriate commercial agents, or thermally, using water at 65°C–100°C or steam [111]. Sanitation protocols should be developed under veterinary consultation to minimize disease risk, especially in quarantine and animal holding areas [112].

Following the One Health approach, eco-friendly alternatives to common chemical products should be used for both outdoor and indoor sanitization. Both natural and synthetic disinfectants, as well as ultraviolet radiation are available for environmental disinfection. These methods have variable efficacy, which may depend on factors such as concentration, exposure time, and biofilm formation [110, 116–119].

6.5 | Vaccination Against Leptospirosis

One report described successful control of a leptospirosis outbreak in a captive population of squirrel monkeys using an in-house inactivated vaccine of *Leptospira interrogans* serovar copenhageni. The monkeys received two subcutaneous doses administered 3 weeks apart. Although no challenge experiment was performed, natural re-infection was not observed for several consecutive years, possibly due to adequate vaccine protection [12]. No other reports exist on the use of vaccines in other NHPs.

While commercial vaccines are available globally for humans, cattle, swine, and dogs, they are only partially effective in these species. This limited efficacy is likely attributable to the serovar-restricted nature of vaccine-induced immunity and the potential presence of local serovars not covered by commercially available vaccines [120, 121]. Currently, a clinical trial is underway involving NHPs in zoos in The Netherlands, utilizing Nobivac L4 (Intervet International B.V., Boxmeer, The Netherlands), an inactivated vaccine designed for vaccination of dogs (H. van Bolhuis, personal communication, May 2025). Future research may focus on assessing the efficacy of commercial vaccines in NHPs and exploring the extent of cross-protection against various serogroups.

7 | One Health Considerations and Surveillance

The interplay of urban encroachment on wild NHP populations, poverty, inadequate diagnostic and veterinary facilities, a lack of knowledge regarding zoonotic diseases, and a disproportionate impact of climate change on local environments significantly increases the overall risk of leptospirosis in economically disadvantaged areas [18, 49, 122, 123]. An integrative approach tailored to the specific local context is essential for effective prevention and intervention [124]. Addressing only one or a few of these risk factors may prove insufficient if the broader One Health aspects of the leptospirosis problem are not adequately addressed.

In the context of zoological institutions, an integrative plan should aim to simultaneously protect NHPs, visitors, and animal care staff from exposure to leptospires [12]. Not only does this include physical measures, e.g., the provision of appropriate personal protective equipment (PPE) to staff, it also involves the education of the public and staff about zoonotic risks. Interdisciplinary collaboration is needed between veterinarians, epidemiologists, ecologists, and local communities. Institutional policies should at least comply to national legislation, as leptospirosis is a notifiable disease is some countries. In addition, moving animals between wild and zoo-or rescue setting should include screening and quarantine to prevent cross-contamination [50].

In addition, ecotourism may pose public health risks by facilitating zoonotic pathogen transmission. Particularly when close and unregulated contact between tourists, (semi)wild animals in potentially contaminated environments is allowed. Therefore, incorporating ecotourism management into comprehensive One Health frameworks is crucial to reducing health risks, protecting wildlife, and promoting responsible tourism [125, 126].

A One Health approach should be adopted when it comes to disease control, epidemiology, and the construction of a surveillance program, as leptospires may be detected in humans, animals, and the environment [49, 110, 127]. For surveillance purposes PCR and 16S rRNA gene sequencing techniques should be used as primary diagnostics and alternatively, though with lower accuracy, DFM to detect *Leptospira* sp. [128–132]. The identification and typing of infecting *Leptospira* spp. can provide insights into the source of the infection, as certain serovars may be directly or indirectly linked to specific groups of mammalian hosts.

Ongoing surveillance systems should be established for the direct and indirect detection of *Leptospira* spp. in local free-living rodent populations, the environment, humans, NHPs, and other animals within zoological institutions or in the wild. This should also include the monitoring for clinical signs of leptospirosis in animals and staff, testing of suspected NHPs, and postmortem research of dead NHPs. Quantification of *Leptospira* spp. in wildlife reservoirs living in the vicinity of urban freshwater areas will be helpful to understand the eco-epidemiology of leptospirosis and to implement targeted prevention and intervention strategies [110, 127]. Monitoring wild NHP populations is particularly critical, as there is limited data on the prevalence of leptospirosis in the different species. Such monitoring would not only provide valuable insights into the potential zoonotic leptospirosis risk that NHPs pose to nearby communities but may also serve as an early warning system when cases of leptospirosis rise among NHPs. Similar surveillance systems are already in place for the early detection of the yellow fever virus and could facilitate the timely implementation of preventive and control measures [133].

8 | Conclusion and Future Perspectives

This review has identified significant gaps in current knowledge, particularly regarding susceptibility of the different NHP species, treatments, preventive measures, and the need for enhanced diagnostic and surveillance strategies. Increasing rat populations exacerbated by ongoing climate change and urban encroachment highlight the necessity for a One Health approach to disease management. Understanding the epidemiology, transmission dynamics, and clinical manifestations of leptospirosis in NHPs is essential for developing effective prevention strategies.

From an epidemiological standpoint, NHPs are particularly interesting due to their close phylogenetic and geographic relationships with humans. Compared to rodents, however, transmission risks by urine are considered low as NHPs are accidental hosts. Even in confirmed cases, *leptospira* was detected rarely in urine, although an explanation for the limited detection is unclear. In addition, their role as a potential reservoir remains insufficiently investigated. Although transmission via rodents to NHPs is the most important route, transmission between NHPs is underinvestigated and cannot be excluded. More research is needed to investigate the roles of vertical transmission and horizontal transmission.

Current diagnostic insights into leptospirosis strongly advocate the use of both serological and molecular techniques for increased diagnostic accuracy. The combinatorial use of PCR and ELISA evades the diagnostic issue presented by the leptospiral biphasic pathophysiology and allows for an early, more reliable diagnosis. A clear diagnostic approach is desirable; nevertheless, the choice of diagnostic test depends on several factors, including the stage of disease, finances, technical or practical feasibility, and the need for a rapid result [68, 134, 135]. It would be advisable to establish an institutional guideline that considers these factors.

For early detection, point-of-care tests which have been developed for dogs could be useful for NHPs under field conditions. A direction for future research should be the validation of these tests in NHPs. Furthermore, current therapeutic recommendations for NHPs are extrapolated from canine and human protocols, pending validation in NHPs. From a preventative point of view, future research may focus on assessing the efficacy of commercial vaccines in NHP-specific trials.

By fostering collaboration between veterinary and public health sectors, one can improve diagnostic accuracy, treatment outcomes, surveillance, public health integration and ultimately reduce the morbidity and mortality associated with leptospirosis in both NHPs and humans.

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