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Research Article

EXPLORATORY SEROLOGICAL INVESTIGATION OF LEPTOSPIROSIS AND SCRUB TYPHUS IN FIELD RODENTS AND SHREWS OF UTTAR PRADESH, INDIA

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ABSTRACT

Rodent and shrew surveillance is essential for public health because these mammals act as reservoirs for several zoonotic pathogens and contribute to disease transmission through direct contact, environmental contamination and amplification of arthropod-borne infections. This preliminary study investigated the presence of *Leptospira* spp. and *Orientia tsutsugamushi* in rodents and shrews from Bareilly, Mathura and Gorakhpur districts of Uttar Pradesh, India. Fifty animals were captured and serum samples were tested for *Leptospira* antibodies using the microscopic agglutination test (MAT) and for scrub typhus antibodies using the Weil-Felix test. All samples were screened against 12 *Leptospira* serovars by MAT and none showed detectable antibodies, suggesting no serological evidence of exposure. In contrast, one sample from Bareilly and two from Gorakhpur yielded presumptive positive reactions for scrub typhus by the Weil-Felix test. Although Weil-Felix has low sensitivity and specificity, the test has been used in the present study as till date no validated serological assays are available for the detection of scrub typhus antibodies in rodents or shrews. Despite these limitations, the detection of presumptive scrub typhus antibodies is notable, particularly given reported human cases from Bareilly and Gorakhpur district. The low apparent seroprevalence may reflect heterogeneous pathogen distribution, transient antibody responses or diagnostic constraints. The absence of *Leptospira* antibodies may indicate lack of exposure, early infection or true absence of the pathogen. Further studies with larger sample sizes, wider geographic coverage and improved diagnostic methods, including ELISA, indirect immunofluorescence assay (IFA) or molecular approaches are recommended to clarify zoonotic disease dynamics.

Keywords: Chiggers, *Leptospira*, Orientia, Rodents and shrews, Zoonotic diseases

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Zoonotic diseases represent a significant global health burden, particularly in regions where human-wildlife interactions are frequent. More than 60% of infectious diseases affecting humans originate from animals, with approximately 75% of emerging infectious diseases having zoonotic origins (CDC, 2024). Among mammalian reservoirs, rodents are the most species-rich order and play a crucial role in zoonotic pathogen transmission. An estimated 10.7% of rodent species are hosts to zoonotic pathogens, carrying at least 85 known zoonotic diseases (Han *et al.*, 2015). Their ubiquitous presence in both urban and rural settings, coupled with their interactions with livestock and human settlements, makes them a significant contributor in pathogen amplification and transmission (Luis *et al.*, 2013; Dahmana *et al.*, 2020). Zoonotic transmission from rodents can occur directly through bites or inhalation of aerosols containing pathogens from rodent faeces or indirectly through contamination of water and food sources (Rabiee *et al.*, 2018). Rodents could also amplify and transfer diseases transmitted by arthropod vectors. Urbanization and deforestation have been associated with increased human-rodent interactions, elevating disease risk (Rupasinghe *et al.*, 2022).

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Among the numerous zoonotic diseases associated with rodents, leptospirosis and scrub typhus are of particular epidemiological importance due to their extensive geographical distribution, potential to cause both localized and large-scale outbreaks and significant morbidity and mortality. These diseases pose a major public health challenge, particularly in areas with high rodent densities and inadequate sanitation. Leptospirosis is a widespread zoonoses recognized as one of the 17 neglected tropical diseases by the World Health Organization (WHO, 2021). Leptospirosis, caused by pathogenic *Leptospira* spp., is primarily transmitted through direct or indirect contact with water or soil contaminated by rodent urine (Vihol *et al.*, 2024). Scrub typhus is a rickettsial disease caused by a pathogenic bacterium, *Orientia tsutsugamushi* and transmitted by *Leptotrombidium* chigger mites (larva of trombiculid mites) through rodents/shrews as primary animal host. Both diseases exhibit a broad spectrum of clinical manifestations, ranging from mild febrile illness to severe complications such as renal failure, meningitis and multi-organ dysfunction. Due to their significant public health impact, implementing robust surveillance systems, targeted preventive strategies and early, accurate diagnostics is crucial for effective disease control and mitigation.

The present study was designed to assess the presence of *Leptospira* spp. and *Orientia tsutsugamushi* in field rodents/shrews of Uttar Pradesh with the ultimate aim to inform control programs in order to prevent spillover of infection to humans.

MATERIALS AND METHODS

Study Design

The study was conducted in selected districts of Uttar Pradesh, India, a state characterized by substantial geographical, ecological and climatic heterogeneity between its eastern and western regions. To ensure representative coverage, districts from both regions were included. Site selection was guided by the availability of laboratory facilities, as transportation of live rodents/ shrews over long distances was not feasible. Among the three selected districts, Gorakhpur (eastern Uttar Pradesh) and Bareilly (western Uttar Pradesh) were included due to the occurrence of reported human cases in the recent past, while Mathura district was selected to broaden regional representation. This approach minimized sampling bias while balancing logistical feasibility and epidemiological relevance.

Uttar Pradesh exhibits marked agro-climatic diversity due to variations in rainfall, soil types and topography. Based on rainfall and soil characteristics, the state is divided into multiple agro-climatic zones ranging from the humid north-eastern plains to comparatively drier central and western plains. Gorakhpur district, located in the north-eastern plains, experiences relatively high annual rainfall and humid conditions, with extensive alluvial soils that retain moisture during and after the monsoon. In contrast, Bareilly district lies within the mid-western/ western plains and is characterized by moderate rainfall, a sub-humid climate and loamy to sandy loam soils with better drainage. Mathura district represents an additional agro-climatic setting within the broader plains system of the state. These differences in rainfall, soil moisture and landscape influence surface water availability and habitat conditions relevant to the environmental survival of *Leptospira* spp. and the ecology of scrub typhus vectors.

Sample Collection

The present study was conducted as a pilot investigation to generate baseline data from selected sites under logistical and ethical field constraints. A total of 50 field rodents and shrews were captured from Bareilly, Mathura and Gorakhpur districts between August and October 2024, including 17 samples from Gorakhpur, 19 from Bareilly and 14 from Mathura. No formal statistical method was employed for sample size calculation due to the exploratory nature of the study.

Trapping was carried out using Sherman collapsible live traps placed in peri-urban areas and human dwellings. Traps were baited with peanut butter and dried coconut to enhance capture efficiency and were set in the evening and checked the following morning.

Ethical Approval and Sample Processing

The study was approved by the Institutional Animal Ethics Committee (IAEC), ICAR-Indian Veterinary Research Institute (F. No. 26–1/2023–24/JD(R)/IAEC), for trapping rodents and shrews. Captured animals were anesthetized using isoflurane and blood samples were collected by cardiac puncture following standard ethical and biosafety guidelines.

Blood samples were collected in clot activator tubes and immediately placed in insulated ice boxes to maintain the cold chain (2-8 °C) during transportation to the laboratory. Serum was separated shortly after collection by centrifugation at 5,000 rpm for 10 minutes. The separated serum samples were aliquoted to avoid repeated freeze–thaw cycles and stored at -20 °C until further serological analysis. All procedures were carried out following standard biosafety and quality control practices to ensure sample integrity.

Statistical Analysis

Regional differences in the occurrence of scrub typhus antibodies among districts were evaluated using Fisher's exact test due to small sample sizes and low expected cell counts. A p-value of <0.05 was considered statistically significant.

Microscopic Agglutination Test

The microscopic agglutination test (MAT) is the reference serological method for the detection of antibodies against *Leptospira* spp. (Levett *et al.*, 2001). It employs a panel of live *Leptospira* serovars representing circulating strains and allows serogroup-level identification when an appropriate antigen panel is used.

MAT was performed following standard procedures recommended by the World Organisation for Animal Health (WOAH, 2023). Validated positive and negative control sera obtained from the *Leptospira* Laboratory, ICAR–Indian Veterinary Research Institute (IVRI), were included in each assay to ensure reliability and consistency of results. An initial serum dilution of 1:50 was prepared by mixing 20 µL of test serum with 980 µL of phosphate-buffered saline (PBS). From this dilution, 50 µL was dispensed into wells of a 96-well microtiter plate and mixed with an equal volume (50 µL) of live *Leptospira* antigen, resulting in a final screening dilution of 1:100.

A panel of 12 *Leptospira* serovars- Australis, Autumnalis, Ballum, Patoc, Pomona, Canicola, Icteroha-

emorrhagiae, Hardjo, Hebdomadis, Grippotyphosa, Javanica and Tarassoviwas used as antigens. Plates were incubated at 37 °C for 2-4 hours, after which agglutination was examined under a dark-field microscope using a 10X objective and 10X eyepiece (total magnification 100X). Samples were assessed for the presence or absence of visible agglutination at the screening dilution.

Weil-felix Test

The Weil-Felix is an inexpensive and widely available serological test. It detects antibodies to alkali-based carbohydrate antigens shared by certain *Rickettsia* spp. and *Proteus* spp. (*P. vulgaris* OX19, OX2 and *P. mirabilis* OXK). The OXK strain specifically agglutinates with sera having antibodies against *O. tsutsugamushi*, aiding in the diagnosis.

The Weil Felix test was performed using the rapid slide agglutination method with OXK antigen (Tulip Diagnostics, Goa, India) to detect antibodies specific to *O. tsutsugamushi* for scrub typhus (Rashmi *et al.*, 2015). A drop of the test serum (50 µL) was placed on a clean glass slide, followed by the addition of an equal volume of the OXK antigen. The mixture was gently rotated on the slide for 1-2 minutes to allow agglutination to occur. The presence of visible clumping indicated a positive reaction, suggesting the presence of antibodies against *O. tsutsugamushi*. Due to the pilot nature of the study and non-availability of standardized positive and negative control sera, assay interpretation was based on visible agglutination at the recommended serum dilution, as per established Weil-Felix test guidelines.

RESULTS AND DISCUSSION

A total of 50 small mammals, comprising 26 rats and 24 shrews, were captured during the study period, including 19 samples from Bareilly, 17 from Gorakhpur and 14 from Mathura. Of the 50 serum samples analysed, three (3/50) showed seroreactivity against *O. tsutsugamushi* using the Weil-Felix test (Fig. 1). Scrub typhus antibodies were detected in 6% of samples, with a 95% confidence interval of 2.0% to 16.0%. Among the positive samples, one was obtained from Bareilly district and two from Gorakhpur district. Although scrub typhus positive samples were detected in Bareilly and Gorakhpur but not in Mathura Fisher's exact test showed no statistically significant difference in the occurrence of scrub typhus antibodies among the three districts ($p = 0.5929$). Notably, human cases of scrub typhus have also been reported from these regions, highlighting the relevance of continued surveillance of reservoir hosts to prevent potential spillover of infection to humans. None of the samples tested positive for antibodies against *Leptospira* spp. when screened using the microscopic agglutination test (MAT) with a panel of 12 serovars.

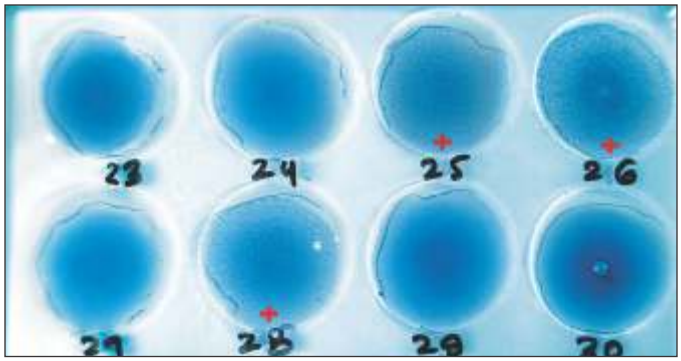

Fig. 1. Representative results of the Weil-Felix slide agglutination test showing visible agglutination (positive) and smooth suspensions (negative) with OXK antigen.
(Samples numbered 25, 26 and 28 show visible agglutination indicating positive reactions, while rest samples show negative reactions.)

Rodent and small-mammal surveillance represents a critical component of public health programs due to the role of these animals as reservoirs for a wide range of zoonotic pathogens. Their close association with human habitations, adaptability to diverse environments and high reproductive rates facilitate pathogen maintenance and transmission. Leptospirosis, caused by *Leptospira* spp., and scrub typhus, caused by *Orientia tsutsugamushi*, are important zoonotic diseases with significant public health implications in India. Serological assays are often employed for surveillance purposes as cost-effective and practical tools, particularly in resource-limited settings, although they possess inherent diagnostic limitations.

In small mammal reservoir hosts, antibody responses to certain zoonotic pathogens may be short-lived or decline rapidly over time, particularly during asymptomatic or subclinical infections (Vernel-Pauillac *et al.*, 2021). As a result, serological assays may fail to detect antibodies depending on the timing of sampling relative to exposure. Thus, the low detection rate observed in our study may be attributed to the patchy distribution of the pathogen, transient antibody responses in reservoir hosts and the inherent limitations of the Weil-Felix test, which is known to have low sensitivity (43.3%) (Prakash *et al.*, 2006). Currently, no standardized or validated serological assay is routinely available for detecting antibodies against *O. tsutsugamushi* in rodent reservoirs. Advanced diagnostic methods such as ELISA or IFA are mainly optimized for human samples and their performance characteristics in rodents/shrews remain inadequately defined. Consequently, despite its well-recognized limitations, the Weil-Felix test has historically been used in preliminary ecological and field surveillance studies involving animal hosts.

Given the limited sample size and restricted geographic coverage, the findings of the present study should be interpreted with caution and are not intended to represent statewide prevalence. In this investigation, 6%

of the sampled small mammals showed seroreactivity to *O. tsutsugamushi*. In India, outbreaks of scrub typhus in humans have been documented across northern, eastern and southern regions (Mathai *et al.*, 2001; Kamarasu *et al.*, 2007; Mahajan *et al.*, 2008; Sharma *et al.*, 2005). Despite its endemicity, scrub typhus continues to be underdiagnosed due to nonspecific clinical manifestations, limited access to advanced diagnostic facilities and inadequate awareness among high-risk populations (Bakshi, 2007).

Rodents are well-recognized reservoir hosts of *Leptospira* spp. and play a significant role in the transmission of leptospirosis to humans and domestic animals (Twigg *et al.*, 1969). Numerous studies worldwide have highlighted the importance of rodents in leptospiral ecology. For instance, a study from Vellore district, Tamil Nadu, reported seropositivity in 42% of trapped rats (Vimala *et al.*, 2014), while a systematic review by Gupta *et al.* (2023) reported exposure to rats in 67% of leptospirosis cases. In contrast, none of the small mammal sera in the present study tested positive for *Leptospira* antibodies using MAT. This absence of seroreactivity may reflect low or absent exposure in the sampled areas, early-stage infections with antibody levels below the detection threshold, or regional variation in circulating serovars. While the present findings suggest a low burden of leptospirosis in the sampled sites, broader investigations employing sensitive diagnostic tools and expanded geographic coverage are warranted.

Despite limitations related to sample size and spatial coverage, the present study underscores the importance of continuous surveillance of rodents and other small mammals for zoonotic pathogens. Even low levels of pathogen detection in localized areas may have important implications for public and animal health. Future studies should incorporate larger, statistically powered sample sizes, precise host species identification and contemporary diagnostic approaches such as ELISA, immunofluorescence assays or molecular techniques to enhance understanding of zoonotic disease dynamics and support effective prevention strategies.

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