UNIVERSIDADE DE LISBOA

FACULDADE DE MEDICINA VETERINÁRIA

IDENTIFICATION OF HOTSPOTS FOR DISEASE TRANSMISSION IN FREE-ROAMING DOMESTIC DOG POPULATION IN UGANDA, INDONESIA AND GUATEMALA

JERÓNIMO QUEIROGA FREUDENTHAL

ORIENTADOR(A):
Doutora Salome Dürr

COORIENTADOR(A):
Doutor Virgílio da Silva Almeida

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Identificação de áreas de alto contacto, relevantes para transmissão de doenças na população de cães domésticos errantes no Uganda, Indonésia e Guatemala

Resumo

A raiva, uma doença zoonótica e endémica doença com grande impacto na saúde pública de diversas civilizações, é atualmente quase sempre transmitida através de interações com cães domésticos errantes em territórios humanos. Este estudo examina os padrões espaciais e temporais dos contactos entre cães em três localizações urbanas distintas, Soroti (Uganda), Habi (Indonésia) e Poptún (Guatemala). Envolveu a colocação de colares de rastreamento em 193 cães, cruzando dados de geolocalização e de contactos. A análise espacial recorreu a estimativas de densidade de *kernel*, evidenciando a distribuição dos pontos de *GPS* e dos contactos entre cães errantes, e o seu rácio. Foram observadas variações interessantes na relação entre a densidade de contactos e a densidade de *GPS* nos diferentes dias e períodos de estudo. Um modelo de regressão linear analisou as dependências entre rácio de densidade contacto:GPS e os dias de estudo e os períodos de tempo. A dispersão espacial foi variável, sendo maior e mais heterogénea em Poptún na Guatemala. Os padrões temporais revelaram um aumento acentuado das taxas de contacto durante os fins-de-semana, particularmente nas sextas-feiras. A consistência espácio-temporal dos padrões das áreas de alto contacto (*hotspots*) revelou-se estável, mas limitada no tempo, e possivelmente influenciada por fatores ambientais e humanos. A comparação entre localizações reforçou o papel dos fatores e infraestruturas na distribuição das zonas de contacto entre os cães. Os resultados obtidos reforçam a necessidade de melhor compreensão sobre o comportamento de deambulação dos cães errantes para um controlo mais eficaz da raiva. A identificação de *hotspots* de contacto fundamenta estratégias de intervenção direcionadas e enfatiza a importância do contexto local. A consistência na sobreposição de infraestruturas com *hotspots* nos diferentes locais, sugere a existência de atrações compartilhadas pelos cães, enfatizando a importância de adequar as estratégias aos contextos de intervenção. Esta investigação contribui com informações úteis sobre a complexa dinâmica espacial e temporal dos contactos dos cães errantes em regiões endémicas de raiva, urbanas ou semiurbanas. Reconhecer estes padrões e compreender as influências locais na dinâmica do cão errante são passos fundamentais para otimizar os esforços de controlo da doença e avançar com o objetivo de erradicação da raiva até 2030.

Palavras-chave: Cães errantes domésticos; Raiva humana transmitida por cães; Taxas de contacto; KDE; Análise espaciotemporal.

Identification of hotspots for disease transmission in free-roaming domestic dog population in Uganda, Indonesia and Guatemala

Abstract

Rabies is a zoonotic disease with major impact in the public health of several civilizations. Nowadays, it is often perpetuated by interactions of people with free-roaming dogs (FRD) in human territories. The intricate dynamics of these interactions, particularly in regions marked by substantial human population growth, call for a detailed understanding in dog roaming behaviour. This study examined the spatial and temporal patterns of FRD contacts in three distinct urban study sites, Soroti (Uganda), Habi (Indonesia), and Poptún (Guatemala), each presenting unique contexts.

The study involved the collaring of 193 FRD, merging their GPS and dog-to-dog contact datasets. Spatial analysis employed kernel density estimates, unravelling the dispersion of GPS fixes and contacts. Temporal analysis showed intriguing variations in the Contact:GPS density ratio across different study days and time periods. Linear regression models probed dependencies on study days and time periods.

Spatial dispersion was diverse between study sites, with Poptún in Guatemala displaying the most heterogeneous and extensive area coverage. Temporal patterns unveiled a marked surge in contact ratios during weekends, particularly on Fridays. Spatial-temporal consistency in contact hotspot patterns revealed stable, yet limited in time, and likely influenced by environmental and human factors. Cross-location analysis underscored the role of local factors and infrastructures in shaping contact distribution in FRD.

The study findings enlighten the critical need for a better understanding of FRD roaming behaviour, for example for effective control of rabies. Identification of contact hotspots supports targeted intervention strategies and emphasizes the importance of local contexts. The consistency in overlapping infrastructure within contact hotspots across locations suggests shared attractions for dogs, emphasizing the significance of context-specific strategies. This work thus advocates for tailored control and surveillance strategies, acknowledging the inherent heterogeneity in dog behaviour across diverse settings. Recognizing these patterns and understanding local influences on FRD dynamics are pivotal steps toward optimizing disease control efforts and advancing the goal of rabies eradication by 2030.

Keywords: Free-roaming dogs; Dog-mediated rabies; Contact rates; KDE; Spatiotemporal analysis.

Resumo alargado

Identificação de áreas de alto contacto, relevantes para transmissão de doenças na população de cães domésticos errantes no Uganda, Indonésia e Guatemala

O estudo obteve aprovação ética para cada local por meio de pedidos separados. As aprovações foram concedidas pela Comissão de Ética Animal da Faculdade de Medicina Veterinária da Universidade Nusa Cendana, na Indonésia, pelo Comitê Internacional de Cuidado e Uso Animal da UVG, na Guatemala, e pelo Conselho Nacional de Ciência e Tecnologia de Uganda. A recolha de dados ocorreu em Poptún (Guatemala), Soroti City (Uganda) e Habi (Indonésia) durante períodos específicos em 2018 e 2019.

Foi delimitada uma área de 1 km² em cada local usando o Google Maps, e foram procurados domicílios com cães. Foram realizadas entrevistas para a obtenção do consentimento dos residentes adultos e preenchimento de um questionário que abrangia os dados do proprietário e do cão, bem como o modo de vida e condições do cão. Os dispositivos de sensores de contacto geolocalizados, desenvolvidos pela Bonsai Systems, foram colocados nas coleiras para registar os contactos (eventos de proximidade) e coordenadas de GPS. Os dois conjuntos de dados foram combinados com base no ID do dispositivo e no carimbo de data/hora, permitindo a correspondência de contactos com localizações GPS com uma janela de tempo de dois minutos. A limpeza dos dados incluiu parâmetros como a velocidade, HDOP e ângulos de trajetória dos cães.

A análise espacial envolveu o mapeamento dos contactos geolocalizados em células hexagonais. A Estimativa de Densidade Kernel (KDE) foi utilizada para identificar áreas onde a concentração de contactos e onde a proporção de contactos para pontos GPS era elevada. A análise temporal categorizou os dados em 12 períodos de tempo, e um modelo linear generalizado avaliou a influência do período de tempo e do dia do estudo na proporção de densidade Contacto:GPS. A variável de resultado foi convertida para o log natural devido a uma assimetria à esquerda na distribuição da proporção.

A análise espacial revelou áreas de contacto entre cães, e a análise temporal procurou identificar períodos em que os cães se encontravam mais propensos a estar em contacto. Os resultados foram visualizados em mapas com o OpenStreetsMap e explorados recorrendo a gráficos boxplot. Equipas locais foram consultadas para identificar estruturas subjacentes às localizações identificadas de maior concentração.

Em suma, o estudo realizou uma análise abrangente do comportamento de cães errantes em três locais urbanos. A integração de dados de contacto geolocalizados e

coordenadas GPS proporcionou informação sobre padrões temporais e espaciais de contactos entre cães. Estas descobertas contribuem para a compreensão da dinâmica das populações de cães errantes, com implicações na saúde pública, no bem-estar animal e na transmissão de doenças.

A análise espacial revelou uma dispersão robusta de coordenadas GPS em todos os locais estudados, sugerindo uma ausência de barreiras para a movimentação dos cães. Poptún apresentou a cobertura mais extensa em comparação com outros locais. A suavização dos dados espaciais com recurso a KDE identificou distribuições de densidade não aleatórias. Foram identificadas áreas onde os cães estavam frequentemente presentes e onde contactavam entre si.

A análise temporal revelou padrões interessantes na proporção de densidade Contacto:GPS. Em Soroti, as horas diurnas exibiam proporções mais baixas, aumentando à noite, sugerindo assim, um padrão de contacto noturno. Curiosamente, durante os fins de semana e as sextas-feiras, os rácios de contacto em todos os locais foram superiores, indicando um padrão semanal possivelmente relacionado com ajuntamentos e atividades comunitárias humanas.

O estudo explorou modelos de regressão linear para cada local, avaliando a relação entre o rácio de contacto e os dias e períodos de tempo do estudo. Em Habi, por exemplo, a proporção aumentou entre o amanhecer e o meio-dia, exibindo uma queda no período consecutivo e aumentando novamente conforme o dia avançava.

Ao combinar as análises mencionadas, a análise espaço-temporal forneceu informação valiosa sobre a consistência de padrões de pontos de maior contacto ao longo dos dias e períodos de estudo. Muitas vezes, os pontos de maior contacto estendiam-se por mais do que um dia, indicando padrões de comportamento estáveis possivelmente influenciados por fatores ambientais e humanos. Comparando a distribuição de pontos de maior contacto entre as localizações, enfatizou-se o papel de fatores locais no condicionamento do comportamento de cães.

A sobreposição de infraestruturas com pontos de maior contacto variou entre os locais. Em Soroti, foram identificadas escolas, levantando preocupações sobre o risco de transmissão de raiva em crianças. Em Habi, os pontos de maior contacto concentravam-se em áreas de vegetação e pontos turísticos. Poptún apresentou um caso variado, com pontos de maior contacto próximos de blocos residenciais, escolas, clínicas veterinárias e áreas florestais fora do planeamento da cidade.

Este estudo reforça a necessidade de uma avaliação detalhada dos fatores que influenciam o comportamento dos cães. A compreensão de padrões espaço-temporais, a consistência de pontos de maior contacto e o estabelecimento de associações entre infraestruturas pode contribuir para o desenvolvimento de estratégias de intervenção direcionadas. Estas incluem a otimização de campanhas de vacinação, a implementação de iniciativas de sensibilização e de protocolos de vigilância com base em demografias e locais específicos.

O estudo fornece uma compreensão detalhada da dinâmica de cães errantes, enfatizando a importância de considerar aspectos espaciais e temporais na análise do comportamento dos cães. As descobertas têm implicações para estratégias de controle de doenças, alocação de recursos e intervenções direcionadas em ambientes urbanos com populações crescentes de cães errantes.

Foram reconhecidas várias limitações. Em primeiro lugar, a recolha de dados decorreu ao longo do tempo e em vários locais. Como tal, o impacto do clima no comportamento não foi tido em consideração, podendo ter influenciado tanto a categorização temporal como a espacial. Por exemplo, Soroti e Habi foram estudados durante a estação seca, e Poptún durante a estação das chuvas.

A representação parcial da população de cães errantes constitui uma limitação crucial, já que nem todos os cães foram observados nos locais. Consequentemente, os resultados devem ser vistos como um subconjunto, ao invés de uma representação abrangente da população total de cães errantes neste locais.

Além disso, foram registados dados incompletos relativos aos primeiros e últimos dias do estudo, devido ao processo demorado de colocação e remoção das coleiras, constituindo um desafio durante a análise. Apesar desta restrição, optou-se por incluir esses dias, visando manter a significância estatística.

A compreensão da dinâmica dos cães requer uma abordagem holística que considere fatores ambientais, sociais e económicos. A investigação da correlação entre os padrões de movimento de cães e práticas comunitárias, juntamente com iniciativas e gastos governamentais, pode fornecer uma compreensão mais abrangente. Além disso, o controlo das áreas de maior contacto podem reduzir significativamente a transmissão urbana, monitorizando aglomerações fora do planeamento urbano, para potenciais eventos de *spill-over*.

Este estudo introduz uma abordagem observacional inovadora, de influência mínima, para revelar locais de alto número de contactos entre cães errantes, evidenciando as complexidade espacial e temporal em regiões endémicas de raiva. A consistência espaço-temporal nos padrões de locais de maior contacto evidenciou um comportamento estável influenciado por fatores ambientais e humanos. Tal pode servir de auxilio no planeamento de intervenções direcionadas com uma alocação eficiente de recursos. A análise entre locais realçou o papel de fatores locais e infraestrutura humana na formação de *hotspots* de cães errantes, revelando consistência apesar das diferenças urbanas.

Os padrões espaciais e temporais revelados destacam a necessidade de estratégias personalizadas, reconhecendo a heterogeneidade inerente no comportamento de cães em diferentes ambientes. Em conclusão, este estudo contribui para a nossa compreensão das dinâmicas complexas da ecologia de cães errantes em regimes urbanos, defendendo abordagens de controle e vigilância personalizadas para otimizar os esforços de controle de doenças e trabalhar em direção à ambiciosa meta de erradicação da raiva até 2030.

Palavras-chave: Cães errantes domésticos; Raiva humana transmitida por cães; Taxas de contacto; KDE; Análise espaciotemporal.

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List of acronyms and abbreviations

ABLV	Australian Bat Lyssavirus
CDC	Centers for Disease Control and Prevention
CSV	Comma Separated Values
EBLV	European Bat Lyssavirus
FAO	Food and Agriculture Organization of the United Nations
FRDD	Free Roam Domestic Dog
GARC	Global Alliance for Rabies Control
GCS	Geolocated Contact Sensors
GLM	Generalized Linear Model
GPS	Global Positioning System
HDOP	Horizontal Dilution of Precision
KDE	Kernel Density Estimate
NA	Not Applicable
NTD	Neglected Tropical Disease
ORV	Oral Rabies Vaccines
PAHO	Pan American Health Organization
PEP	Post-exposure Prophylaxis
PrEP	Pre-exposure Prophylaxis
QGIS	Quantum Geographic Information System
RABV	Rabies Virus
RIG	Rabies Immune Globulin
RSSI	Received Signal Strength Indicator
UAR	United Against Rabies
UTM	Universal Transverse Mercator
VPHI	Veterinary Public Health Institute, University of Bern
WGS84	World Geodectic System 1984
WHO	World Health Organization
WOAH	World Organization for Animal Health

1. Internship report

During the period from September 15, 2022, to December 15, 2022, the intern engaged in an extensive and comprehensive problem-based course under the guidance of Professor Telmo Nunes at the Faculty of Veterinary Medicine, University of Lisbon. This course was designed with a dual objective: to increase the proficiency in R programming, related tools, and quantum geographic information system (QGIS) for spatial data manipulation, and to provide students with invaluable insights into the fields of Epidemiology and Veterinary Public Health. Throughout the 500-hour course, the intern encountered a diverse range of challenges, including data processing, statistical analysis, and reporting, with a primary focus on conveying the epidemiological status of specific diseases. Students were actively encouraged to explore the most suitable and up-to-date solutions, all while benefiting from Professor Telmo's expert guidance.

Following the traineeship coursework, the journey continued with a curricular internship from January 15th to April 15th of the same year at the Veterinary Public Health Institute (VPHI), University of Bern. This immersive experience was conducted under the mentorship of Professor Doctor Salome Durr and PhD/residency student Laura da Silva, totalling 600 hours. The early phase of the internship involved discussions on the potential applications of the datasets, topics for further investigation, and for publication.

The intern actively participated in weekly meetings throughout the internship that allowed him to track his project's progress and breakthroughs. The focus of the internship centred on data manipulation, culminating in the development of an adaptable R Markdown document that could be applied to different locations, considering the similarity of the datasets collected. Finally, this tool was then adapted and applied to several locations, being Uganda, Guatemala, and Indonesia the chosen for the thesis. Additionally, the intern was given the opportunity of attending a condensed QGIS course, presented by the geography department in University of Bern that contributed greatly to this work, and his knowledge of spatial data.

In parallel with the master's thesis work, the intern had the privilege of following other projects within the institute, which spanned a diverse and captivating range of research endeavours. The knowledge in Epidemiology and Veterinary Public Health was deepened by participating in journal club meetings hosted by residency and PhD students addressing critical examination and discussion of cutting-edge publications within the area. Additionally, weekly VPHI webinars were attended, where accomplished speakers from various backgrounds shared their insights and expertise, each with remarkable projects.

As the internship progressed, the opportunity to contribute to other projects was presented. This included active participation in Laura da Silva's scoping review on free-roaming dog enumeration methodology, a crucial aspect, but not exclusive, of population management and intervention planning for rabies control. Furthermore, I was given the opportunity to participate in a consultancy project requested by the government. This project revolved around assessing the utility of the forced swim test in evaluating the effectiveness of antidepressant medication, with implications for potential regulatory actions. Finally, I was invited to join another scoping review, which focused on Knowledge, Attitudes, and Practices regarding rabies on a global scale, aiming to unravel human perceptions of the disease across different regions.

The scoping review projects were managed concurrently with the thesis writing process, both conducted remotely. We actively engaged in weekly meetings concerning thesis writing while contributing to these additional research projects. The culmination of the internship and cooperation with the VPHI resulted in co-authorships for the scoping reviews. Finally, the contents of the master thesis are to be published, following the submission and review processes.

2. Introduction

2.1. The controversial contract

Humans and dogs have enjoyed coexistence and the benefits of a symbiotic relationship for thousands of years, with evidence implying that this process was initiated by European hunter-gatherers 18,800 to 32,100 years ago (Dayan 1994; Thalmann et al. 2013). Interestingly, remains from a Roman dog dating back to 169 BC – 8 AD were unearthed, along with its likely owner, in 2007, and showed similar craniometrics to the modern-day brachycephalic breeds, such as French Bulldog or Pekingese dog (Onar et al. 2023). Such discoveries may suggest humans' relationships with dogs have remained, as we currently perceive them, for 2,000 years. Likewise, it is speculative what has brought upon this companionship, but anywhere in the world, a walk outside is enough to see it has transcended eras.

Domestication practices started with the dog and enabled humanity to farm, herd, and hunt and prosper, but humans could not fully grasp the underlying terms of this practice. Dogs are a potential source of direct injury to humans, mainly children, and other domestic animals or wildlife. Their unsupervised and uncontrolled roam may cause road accidents, noise pollution, shedding of parasites and microorganisms, and pose as a source of zoonotic diseases (Butcher and Keuster 2013).

A zoonosis, or anthropozoonosis, as defined by the World Health Organization (WHO), is a disease that is naturally transmissible from vertebrate animals and humans. Jones et al. (2008) found that between 1940 and 2004, about 60% of the emerging infectious diseases were zoonotic and it has been estimated that 99% of all human cases of zoonotic disease infections arises from domesticated animals, be it through direct contact, contaminated food and water consumption or invertebrate vectors (Weiss and Sankaran 2022)

Among the notable examples of zoonotic diseases transmitted by dogs is rabies, which has had an unaccounted impact on humans over the centuries. Evidence of this ancient infectious and deadly disease can be traced back to Sumerian and Akkadian literature dating back 4,000 years (Yuhong 2001). Presently, dog rabies continues to be a threat, claiming responsibility over above 99% of reported human rabies cases. Rabies causes an estimated 59,000 human deaths annually and economic losses of 8.6 billion USD per year (Hampson et al. 2015; Fooks et al. 2017). Understanding and managing the risks associated with zoonosis such as rabies remains crucial in preserving the longstanding and beneficial relationship between humans and dogs.

2.2. Rabies, a brief review

Rabies is an acute viral progressive encephalomyelitis, caused by a lyssavirus within the Lyssavirus genus of the Rhabdoviridae family. Once considered a single pathogen, or ailment, it has been proposed that at least seven genotypes are responsible for the disease, and it is now understood that rabies results from at least seventeen different lyssaviruses species (Rupprecht et al. 2020). The classical rabies virus (RABV) genotype 1, stands as the most widely distributed and prevalent variant in the Americas, thus far.

Rabies virus is considered the prototypical neurotropic virus, with a small negative-strand RNA genome, encoding 5 proteins: the ribonucleoprotein, the functional template for viral replication; the polymerase and phosphoprotein, both parts of the polymerase complex; the matrix protein, the connection between the capsid and the virion membrane; the glycoprotein, externally and tightly disposed, like a trimmer, for cell invasion (Tordo et al. 1986; Conzelmann et al. 1990; Schnell et al. 2010). Despite significant technological and clinical advancements, it remains nearly invariably fatal once symptoms become evident. However, debatably, some therapeutics have shown some results (Willoughby et al. 2005; Jackson 2013a).

It is important to note that although all mammals are susceptible to rabies, not all species are able to sustain the virus within their populations. The RABV is commonly found across carnivore species, including dogs, foxes, raccoons, vampire bats, and bats, representing grand part of the virus' interface with humans (Hemachudha et al. 2002). Notably, bat-associated genotypes such as Lagos bat virus, Australian bat lyssavirus (ABLV), European bat lyssavirus type 1 and type 2 (EBLV), and Duvenhage, have been isolated from bats inhabiting all continents worldwide, except Antarctica. Contrary to what rabies poses to most mammals, EBLV type 1 seropositive bats may survive for at least 3 years (Serra-Cobo et al. 2002). The Mokola genotype is the exception and was only found in shrews and cats (Kgaladi et al. 2013).

2.2.1. Distribution and economic burden of rabies

The distribution of rabies virus genotypes across the globe is not uniform. While rabies virus is present worldwide with the exception of Antarctica, certain genotypes are confined to certain regions. For instance, the Lagos bat virus, Mokola, and Duvenhage genotypes are exclusive to Africa, whereas, the EBLV are primarily restricted to Eurasia, and the ALBV is predominantly found in Australasia (Warrell and Warrell 2004). This distinct geographical confinement underscores the intricate interplay between viral genetics and regional ecosystems.

Likewise, dog rabies' impact is not evenly distributed throughout the world. While the dynamics of the rabies viruses remain uncertain, it has been suggested that during the 19th Century in Central and Western Africa, and the Americas, European colonial influence and urbanization played a role in spreading the disease. This hypothesis underscores the influence of human activities on disease dynamics and provides a glimpse into the complex coexistence with the virus (Smith et al. 1992; Talbi et al. 2009). Perhaps consequently, the disease is most prevalent in Africa, Asia, and Latin America, with 95% of death occurring in the first two continents. The grand majority of human deaths occur in Africa (36.4%), with the highest per-person death rate taking place in sub-Saharan countries. Asia holds the highest reported count (59.6%), with India leading the trend with 35% of the total global deaths. On the other hand, the Americas carry 0.05% of the burden, out of which, Haiti constitutes 70% of the cases (Hampson et al. 2015).

2.2.2. Pathogenesis

In 1804, Georg Gottfried Zinke injected saliva from a rabid dog into a healthy dog, thus laying the foundation for the understanding of the transmission of rabies (Jackson 2013b). Nowadays, it has been reported and reviewed that during most of the incubation period, the wild-type RABV lies in the bitten muscle in a smouldering, low-replicative state. This leads to a delayed, and highly variable, incubation period (Charlton et al. 1997; Hemachudha et al. 2013).

Once incubation is complete, invasion follows. The entry route was determined by the observation of high viral concentrations at the neuromuscular joints, but not at the sensory and automatic synapsis (Ugolini 2011). It was further observed that RABV bound to the nicotinic acetylcholinergic receptors on the post-synaptic site (Lewis et al. 2000). Interestingly, bat lyssavirus variants were shown capable of replication in epidermal cells in vitro, and patients exhibited higher local neuropathic pain, suggesting progression via sensory skin innervation. Consequently, patients with negligible scratches caused by bats are considered at very high risk of infection (Hemachudha et al. 2002). Following the uptake by the motor endplates, centripetal propagation of the virus occurs via the respective neurons. Shortly after, the correspondent dorsal root ganglia are heavily infected, further spreading the infection via intraneuronal pathways. This way, the infection rapidly spreads to the ipsilateral root ganglia (Charlton et al. 1996). At the same time, retrograde neuronal transport further spreads the pathogen to the brainstem and corticospinal pathways, eventually invading higher-order CNS neurons and causing an encephalitis (Charlton et al. 1996; Ugolini 2011).

As replication takes place in the neuronal cells, the virus is transported to the ventral and dorsal roots ensuing centrifugal spread to extraneural organs via sensory innervations (Charlton et al. 1997; Jackson et al. 1999). This way, the salivary glands are populated and, in association to the neuropathies and consequent behavioural changes including aggressiveness causing bites, the contagion through animal bite is alas made possible.

2.2.3. Clinical presentation

Rabies is characterised, most frequently, by acute behavioural changes and progressive paralysis. It is possible to classify the progression of the disease into two phases, prodromal, and acute neurological phase, though clinical presentation can vary considerably between patients. During the prodromal stage, mammals show vague non-specific signs, such as fever, pruritus, and paraesthesia, with a tendency for rapid evolution. Entering the acute neurological phase, lasting one to four days, the patient exhibits specific signs that allow further characterization into furious and paralytic forms. After the onset of coma, death occurs within one to seven days (Fooks et al. 2017).

Rabies can be further classified into two types: furious and paralytic. The first lasts for 1-7 days and is associated with the involvement of the forebrain. The diseased individual becomes restless and increasingly sensitive to sensory stimuli, develop photophobia, and turn delusional, snapping at apparitions. When the restlessness intensifies, animals tend to roam, further increasing the stimuli they are subjected to, resulting in an increasingly irritable and vicious animal. The culmination of these behavioural changes contributes to heightened aggressiveness, leading to a higher incidence of bites, and consequently, transmission of the disease to other victims. Curiously, some animals, instead, avoid human contact and seek dark or quiet shelters, away from the stimuli (Greene 2014).

The paralytic form of the disease typically emerges 2-4 days after the initial appearance of clinical signs. The paralysis gradually extends throughout the central nervous system. As the infection advances, the brain stem is eventually affected, leading to laryngeal paralysis. This manifests as a change in the tone of the animal's bark, or person’s voice, and excessive drooling or frothing due to the inability to swallow and laboured breathing (Greene 2014). Although their progression is distinct, both forms invariably lead to coma, respiratory failure, and death.

2.3. Rabies’ control

Historically, the everlasting and unaccounted impact of the disease, coupled with human progress in medicine, led Louis Pasteur and his colleagues to successfully inoculate two at-risk patients with a live attenuated vaccine in 1885 (Tarantola 2017). This breakthrough set in motion a series of advancements in vaccine development, creating a pathway for humanity to control and even entertain the idea of eradicating diseases. In the present day, the concepts of vaccination or pre-exposure prophylaxis (PrEP) and post-exposure prophylaxis (PEP) stand as the cornerstones of rabies prevention, rendering this disease 100% preventable (WHO 2018).

2.3.1. A 100% preventable disease

Post-exposure prophylaxis comprises a collection of practices for humans after virus exposure that aim at reducing the viral load invasion and stimulate the immune system for the upcoming infection. Therefore, it is dictated that when the suspicion of virus transmission arises, be it by exposure to saliva, scratches/abrasion, or transdermal bites, one must first wash the wound with soap and flush it for 15 minutes, as soon as possible. This ought to be, given it is a possibility, followed by administration of rabies immune globulin (RIG) if the injury is a lacerative bite-wound or the scratch from a bat (given the risk of bat lyssavirus infection). Lastly, several active vaccination shots are to follow, according to established protocols (WHO 2018).

Full intramuscular vaccination protocol is lengthy and costly (Wieten et al. 2013). However, it has been documented that with a single intramuscular dose, and after exposure to PEP after a year, all patients developed a sufficient antibody response; suggesting that a single dose vaccination followed by PEP may induce life-long immunological memory (Jonker and Visser 2017; WHO 2018). Nowadays, intradermal administration has been further studied and found to be more cost-efficient, due to the abbreviated schedule and smaller dosage (a fifth of the intramuscular dosage) of vaccine required (Mills et al. 2011; Wieten et al. 2013). Nonetheless, access to human vaccination is still often non-affordable or non-accessible, which is responsible for rabies cases in many countries that could be prevented.

The WHO recommends providing PrEP to individuals deemed at high risk of contracting the disease. High risk may result from residing in remote areas of endemic countries with limited access to medical care or living in regions with a high prevalence of dog-mediated rabies. The latter is defined as rabies virus transmitted primarily through domestic dogs' bites, and accounts for 21 476 and 35 172 human deaths in Africa and Asia, respectively (Hampson et al. 2015). This way, the WHO designates part of Africa, with a few exceptions in the south, and a significant portion of South and Southeast Asia as high-risk zones (WHO 2018).

Given the worldly disparities, not all organizational institutions can guarantee the availability of PrEP, or the proper compliance to PEP protocols despite the above-mentioned advances. Concordantly, the developing world remains the most affected, comprising most of human rabies deaths (Knobel et al. 2005). Therefore, rather than only focusing on rabies prevention in humans after exposure, it has been proven more efficient controlling the disease at its source, i.e., at the interface between domestic dogs and humans (Zinsstag et al. 2009; Lembo et al. 2010). According to Cleaveland (2003), attaining a 60-70% vaccination coverage in the dog population may suffice to eliminate rabies in the respective population. This, in turn, has the potential to reduce the incidence of rabies transmission to humans, consequently lowering the demand for PEP.

In this manner, the endeavour to manage and potentially eliminate rabies serves as a prominent illustration of the 'One Health' strategy. This approach involves intervening within animal populations to yield benefits for both, the animal and human health. Beyond its cost-effectiveness, this approach addresses equity concerns by removing socio-economic barriers to accessing rabies prevention medication (Cleaveland et al. 2017). Undoubtedly crucial though it may be centring on PEP as a disease control method falls short of achieving disease eradication. Without meticulous preparation and implementation of widespread vaccination initiatives, low- and middle-income countries find themselves reliant on PEP, restricted as it is. The current body of evidence and research strongly suggests the feasibility of the elimination of canine rabies by mass vaccination of dogs, and therefore eliminating dog-mediated humans' rabies (Cleaveland and Hampson 2017).

Curiously, to reduce costs and increase vaccination coverage, oral rabies vaccines (ORV) previously designed for wildlife were repurposed and adapted for dogs (WHO 2007). This year, the SPBN GASGAS, egg-flavoured ORV, became the first oral vaccine bait that complies with the World Organization for Animal Health (WOAH) international standards. Such breakthroughs shine light on the tripartite goal of eradication, *Zero by 30*, as described below (Bobe et al., 2023).

2.3.2. A neglected disease

The WHO classifies neglected tropical diseases (NTD) as a group of infectious diseases that are mainly prevalent in the tropical areas, affecting more than one billion people. The group comprises parasites, bacteria, fungi, toxins, and viruses, being among the latter, rabies (WHO 2023).

Despite the arsenal of tools developed and the decades of progress and investigation, rabies is still a dire reality for most of the world population, excepting high-income countries. However, human dynamics and conflicts diverts attention and may allow the regress of once effective measures (World Veterinary Association 2023). For this reason, those harbouring political instability and conflict cannot afford to properly report, and consequently, maintain effective surveillance and control of infectious diseases (Wilde et al. 2016).

Various factors including misdiagnoses (Mallewa et al., 2007), deficient surveillance leading to unreliable data, and the absence of effective dog population control, contribute to the classification of rabies as a neglected tropical disease in many affected countries (Warrell and Warrell 1995). Additionally, challenges in providing efficient PEP and vaccination, primarily due to significant institutional opportunity costs, further compound the recognition of rabies as a NTD. It is estimated that human deaths are underreported by a factor of 100-fold in endemic countries (Cleaveland et al. 2002; Taylor and Nel 2015). Due to underreporting of rabies cases, policymakers lack the notion of the extent to which the population bears the burden. Hence, this information gap results in inadequate funding and limited investment, exacerbating the existing epidemiological situation and subsequently escalating the burden even further. This way, a vicious and frustrating cycle is maintained (Taylor et al. 2017).

2.3.3 *Zero by 30*

As progress calls for action, the WHO, WOAH and UN Food and Agriculture Organization (FAO), together with the Global Alliance for Rabies Control (GARC) launched the daring and motivating goal to eradicate dog mediated human rabies in adhering countries by 2030 (Report of the Rabies Global Conference Human Rabies of Dog-Mediated, 2015). Five pillars were defined in this conference:

1. Socio-cultural pillar: stakeholder involvement and awareness are essential in dog ownership and PEP practices.
2. Technical pillar: calling for the development of systems that optimize vaccination and surveillance.
3. Organizational pillar: promoting leadership, coordination, and interdisciplinary One Health approach.
4. Political pillar: attributing due importance to this neglected disease, through proof of burden and appropriate action.
5. Resource pillar: efforts tend to develop in the long run, calling for efficient allocation of resources, through responsible, sustainable, and consistent investment.

2.4. Dogs as a public health liability

Back in 1975 in the USA, Beck (1975) observed a concerning trend where dogs were becoming a subject of social, political, and medical worry. The rapid increase in dog population prompted the realization that greater attention should be given to dog-mediated zoonosis, and strict legislative measures advocated for controlling these population. However, not all countries could afford the attention these issues require, and consequently, it is currently estimated that there are globally over 700 million domestic dogs, 75% of which are considered free-roaming dogs (FRD) (Hughes and Macdonald 2013).

2.4.1. Free-Roaming Dogs

The WOAH defines an FRD, as a dog that is not directly under human supervision or control, be it owned or unowned. This way, they reproduce and roam uncontrollably, leading to overpopulation and a variety of negative consequences.

Concordantly, when high densities of FRD are prevalent, and due to its unavoidable proximity to humans, a number of issues are observed. It is widely acknowledged that human rabies cases are predominantly the result of dog bites, accounting for over 99% of such instances (Fooks et al. 2017). Regarding public health, it was shown by Jiménez et al. (2002) in Spain that controlling *Echinococcus granulosus* in FRD reduced its incidence by 97.2%, justifying the investments made. Regarding animal production, Home et al. (2017) noted in India that dogs targeted small ruminants preferably and their impact was superior to that of wildlife predators. Finally, it was described that wildlife is seriously affected, in this particular case, being responsible for the extinction of 11 species and further endangering another 200, with prospection of worsening, given the raising number of dogs and humans (Doherty et al. 2017; Nayeri et al. 2022).

Although the consequences above described can be put in economic terms, high prevalence of FRD and risk of dog bite directly affects the tourism industry of a country, further deepening the economic burden. Such was observed back when the Centre for Disease Control and Prevention (CDC) issued a warning describing the situation in Bali, where two people contracted the disease and died, and 100 other had been reportedly bitten per day (Focus Taiwan 2014). In Bhutan, Strickland (2013) sought to record tourists' perspective on the matter. Their responses, although variable, highlighted that dogs formed aggressive packs during the night, leading to sleepless nights due to the barking and to fear of being bitten. All this is said to be aided by the lack of law making and veterinary care in the country.

2.4.2. Where FRD roam to

Bhattacharjee and Bhadra (2020) conducted a study aimed at addressing the existing gap in the literature concerning FRD ecology within urban settings in India. This investigation focused on examining the relationships among FRD themselves, between FRD and humans, and their associations with particular areas designated as having either moderate or high levels of human activity. Areas with a moderate level of human activity exhibited stronger connectivity, with humans found to initiate interactions—both positive and negative—more frequently than dogs (Bhattacharjee and Bhadra 2020). This would suggest that a FRD's roam pattern is affected by anthropogenic activity and, consequently, the characteristics in urban areas.

Similarly, Cunha Silva et al. (2022) performed a comprehensive study on the roaming patterns of FRD in both urban and rural environments, spanning Indonesia and Guatemala. The study employed geo-referenced contact sensors for tracking dog's movements. The intriguing outcome of this investigation revealed a consistent choice among FRD for human infrastructure, particularly buildings and roads. This choice persisted regardless of the country or the human population setting, whether rural or urban, suggesting notable preference toward proximity to human-made structures (Cunha Silva et al. 2022).

Beck (1975) in Baltimore and St. Louis, U.S.A. also found that urban FRD could subsist on human disposables and garbage alone. In addition, a photographic study conducted in Brazil unveiled a significant correlation between clusters of FRD and the presence of available food resources (de Melo et al. 2023). Notably, these clusters were particularly concentrated around community feeding sites and commercial food outlets, emphasizing the role of food availability, and human activity, in shaping FRD distribution (Beck 1975; Vanak and Gompper 2009; de Melo et al. 2023).

The above-mentioned findings prompt further investigation into this relationship and the areas FRD show preference over, offering valuable insights for effective management and healthy coexistence.

2.5. The situation of rabies and FRD in Uganda, Indonesia, and Guatemala

In Uganda, from 2001 to 2015, on average 486 humans died every year of rabies, whilst an average of 13,900 human animal bites were registered annually (Masiira et al. 2018). Despite remaining a neglected disease, the Ugandan Ministry of Health and Agriculture counts rabies as one of its seven priority diseases, spending an average of 1.9 million dollars (UGX 7 billion) on rabies management, through PEP and animal vaccination. The true burden of the disease in the country is unknown, due to poor surveillance and underreporting (Omodo et al. 2020). Dog ownership influences the conditions in which the risk presents itself to humans. Disregarding the impact of the disease, the communities', and dog owners' knowledge on and positive perception towards animal vaccination is not widespread (Wallace et al. 2017).

Indonesia ranks fourth highest in Southeast Asia for rabies impact, following India, Bangladesh, and Myanmar. The country notifies 150 to 300 human cases of rabies per year (WHO 2012). The first confirmed case of rabies was reported in 1889, in the Jakarta district. Given human restlessness and globalization, the disease spread through the archipelago.

Concordantly, for the past 20 years the disease is still in an active state of emergence and re-emergence, given illegal and uncontrolled human translocation, along with their dogs (Ward 2014). Flores Island was historically rabies free until 1997 when an outbreak was reported. The phylogenetical analysis suggests it originated from Sulawesi, further highlighting the human mediation of rabies spread (Susetya et al. 2008). Currently, dogs are considered the main reservoir of the disease on the island, being responsible for 19 reported human deaths every year. Governmental veterinary services are vaccinating, culling, quarantining, and running diagnostics in efforts to mitigate this impact (Wera 2017). In 2012, Wera et al. (2015) conducted an investigation on the effectiveness of vaccination campaigns in Flores Island, and found that the overall uptake was low, despite knowledge being considered high (Wera et al. 2015).

Guatemala stands as a hopeful example for Latin America regarding rabies eradication efforts. The government, together with the Pan American Health Organization (PAHO), have been leading vaccination campaigns since 1984, reducing canine rabies incidence by 99% (Vigilato et al. 2013). The work done throughout the years, through calculation of human:dog ratios, FRD population estimates, targeted vaccination campaigns and estimating levels for herd immunity, culminated in three human reported cases per year (Wallace et al. 2017).

Due to their importance in the epidemiology of rabies, several studies were carried out in these countries on FRD. Between 2017 and 2020, a research study investigated FRD encompassing the above-mentioned countries, plus Chad. The complexity of contact networks between FRD, and whether certain factors, such as urban or rural settings, religion, income, or education, were investigated and associated with higher or lower levels of contact rates (Warembourg et al. 2021). This requested for further investigation of the complex socio-economic and cultural influence. To optimize vaccination campaigns, tackling highly connected dogs would theoretically reduce the number of vaccines required, as these would be responsible for the majority of the disease transmission chains in case of an outbreak. This strategy would optimize vaccination, resource allocation, and contribute towards the 2030 goal (Warembourg, Fournié, et al. 2021). On the same accord, home ranges were investigated across different countries (Warembourg, Wera, et al. 2021) and a new methodology of dog enumeration was tested, using unmanned aerial vehicles, essential for any control effort (Warembourg et al. 2020).

Complementary to these studies, the habitats FRD displayed a higher presence, or preference over, in urban and rural areas in endemic countries were also investigated, unveiling high-risk locations and potential ORV drop sites (Cunha Silva et al. 2022).

Additionally, the temporal distribution, or the time windows, of a dogs' daily activity was investigated in Guatemala, and in Switzerland, showing the similarities dogs have despite their markedly different environments (Griss et al. 2021).

Despite the extensive and intricate body of literature within Warembourg's research, one question remained unanswered: the specific locations and times at which FRD would most frequently contact each other, a prerequisite for rabies transmission.

2.6. Study objectives

This project aimed at complementing previous research by identifying specific locations and time windows associated with high contact rates between FRD, and higher likelihood of disease transmission. Consequently, a tool was developed with the goal of aiding decision-makers.

This study was undertaken with four key objectives:

1. **Characterizing spatial roaming patterns**: The primary objective of this research was to characterize the spatial roaming patterns of FRD in urban settings.
2. **Temporal analysis of dog roaming**: Another objective was to perform a temporal analysis of dog roaming behaviour. This included identifying periods during the day and days of the week when FRD exhibit specific movement patterns and contact rates. By examining temporal variations, we sought to indirectly investigate and discuss the factors influencing dog roaming, such as daily routines and human activity.
3. **Identifying contact hotspots**: This objective aimed to identify and delineate contact hotspots among FRD. We aimed to pinpoint, with a high level of resolution, locations where FRD frequently contact each other. By doing so, we intended to contribute for a better understanding of the dynamics of FRD interactions, which is vital to assess rabies transmission risks.
4. **Informing rabies control strategies**: Ultimately, the overarching goal of this study was to provide valuable insights to inform rabies control strategies to the local teams. By characterizing the roaming and contact patterns of FRD, we aimed to generate essential information that could be used to develop targeted and effective rabies control interventions.

Therefore, this study will contribute towards the implementation of effective measures to control FRD population, limit their interactions with each other, humans, and other animals, and reduce the risk of disease transmission. Such information could help to guide targeted vaccination campaigns, determine drop-sites for oral vaccine baits, and educational initiatives.

3. Materials and methods

The study ethical approval was obtained for each location through separate applications. The research was granted permission by the Animal Ethics Commission of the Faculty of Veterinary Medicine at Nusa Cendana University (Protocol KEH/FKH/NPEH/2019/009) in Indonesia, UVG's International Animal Care and Use Committee under Protocol No. I-2018(3) in Guatemala, and by the Uganda National Council for Science and Technology under Protocol NS640 in Uganda.

Data was previously collected in four countries, Indonesia, Uganda, Guatemala, and Chad, in urban and rural locations (Warembourg, Wera, et al. 2021).

For the present study, we selected three urban locations: Poptún, a municipality of El Petén in Guatemala; Soroti City, the capital of the Soroti District in Uganda; and Habi, located in the Sikka Regency of Indonesia. The choice of these locations was driven by the collaborative synergy between the local research teams and ongoing projects, as well as considerations regarding the expected population of FRD inhabiting these areas.

The data was collected from the 31st of May to the 5th of June 2018, in Poptún; from the 27th of July to the 1st of August 2018 in Habi; and from the 23rd to the 28th of January 2019 in Soroti.

For each of the three locations, a 1 km^2 area was delimited using Google Maps, and dog-owning households were sought after within this 1km^2 study sites. Each dog's owner was invited to participate in the study by the local team. After oral or written consent from a resident adult, an interview questionnaire was performed, and the dog was collared with a geolocated contact sensors (GCS) device. In addition, the chance was offered to vaccinate the participants' dog against rabies.

The questionnaire included questions on the demographics of the owner (i.e., ethnicity, religion, age, income, gender) and the dog (i.e., breed, sex, age, reproductive status), standards of living (i.e., running water access, electricity), and dog ownership conditions (i.e., veterinary care, dog confinement, dog utility) (Warembourg, Fournié, et al. 2021).

The exclusion criteria for dogs participating in the study were dogs younger than four months, sick dogs, late-stage pregnant bitches, and dogs marked for slaughter in the following four days (in Indonesia only). Dogs whose necks were too thick for the collar, too nervous or violent, and when the owner consent was absent, or the dog was missing were also excluded.

Bonsai System developed the GCS devices attached to the collars (Bonsai Systems 2020). Each device had a unique ID number and collected two types of data: i) the contacts (or proximity events) between collared dogs up to 4 meters apart through an ultra-high-frequency sensor (Laager et al. 2018). The beacons were sent out every minute and recordings by peer devices was done continuously, and ii) their Global Positioning System (GPS) location through a tracker module collecting a GPS fix every 60 seconds. The two datasets were downloadable after retrieval of the devices (Table 1):

Table 1 – Data collected by GCS devices from free-roaming dogs in Uganda, Indonesia, and Guatemala.

Contacts / Proximity Events	Global Positioning System Fixes
Device ID: Collar identifiers.	Device ID: Collar identifiers.
Timestamp: Year/Month/Day and Hour/Minute/Second of the contact.	Timestamp: Year/Month/Day and Hour/Minute/Second of the GPS fix.
Received Signal Strength Indicator (RSSI): Signal strength.	Horizontal Dilution of Precision (HDOP): Accuracy of the coordinates.
Contact Peer Dog ID: Second dog's ID involved in the proximity event.	GPS Coordinates (WGS 1984): longitude and latitude of the GPS fix in a WGS 1984 projection in decimal format.

The GCS data was fully used, whilst the questionnaire survey data was only used to identify roaming restrictions of dogs (i.e., keeping the dogs chained up or kept in cages).

3.1. Data Processing

For the purpose of data cleaning, analysis, and presentation, R software was used (version 4.2.2), operated in RStudio and Markdown (R Core Team, 2022).

3.1.1. Data cleaning

The GPS data used in this study had been previously cleaned for the works of Cunha et al. (2022). Parameters taken into consideration for data cleaning were HDOP, speed and incidence angle from consecutive GPS fixes, as proposed by Cunha et al. (2022). It was, therefore, implemented a triphasic data cleaning process, where entries were to be excluded if: i) the speed calculated from the dogs' consecutive GPS fixes in time would surpass 20km/h, as it was deemed unlikely that a dog would run that fast for one minute, being probably due to vehicle transportation (Dürr and Ward 2014); ii) the GPS fix has an HDOP of 5, since this is a sign of poor accuracy of the fixes (Dore et al. 2020; Lewis et al. 2007); iii) the angle between two trajectories built by three consecutive GPS fixes was in the upper 0.025 quantile of acute inner trajectory angles, as it was associated with non-realistic animal movement pattern and therefore, a GPS fix error (Shimada et al. 2012).

Additionally, the coordinates were projected into the correspondent Universal Transverse Mercator (UTM) coordinate reference system of the location before further processing to account for the earth's curvature.

3.2. Data Set

As the GCS devices registered data in two different datasets (proximity events and GPS fixes), the cleaned data of the two datasets were merged. Since data entries in both datasets contained a timestamp and device ID, it was possible to match the entries, i.e., to allocate a specific contact to a GPS location. However, contact and GPS fixes were seldom recorded in the exact same instant. To tackle it, a time window of two minutes was created, and a match was reached when contact and the GPS fix of the same dog happened within the same two-minute time window. Not all contacts could be matched with a location, and the non-match was unevenly distributed amongst dogs. There were also some dogs without GPS information. This resulted in a loss of these dogs and thus in a considerable number of contacts with lacking location (Table 2).

Table 2 - Data availability before and after data merge between the two datasets.

Location	Nº of dogs pre-merge	Nº of dogs post-merge	Cleaned GPS fixes (pre-merge)	Contacts data (pre-merge)	Geolocated contact data (post-merge)
Soroti	114	61	88,772	91,941	77,039
Habi	96	66	84,796	91,905	45,750
Poptún	109	62	58,607	194,095	28,193

After matching the contact with the GPS data, the analysis focused on the geolocated contact data and the GPS fixes of the dogs with matched geolocated contacts. The former was used to identify locations where dogs contact each other, whereas the latter was used as a baseline layer for the graphical representation of dog locations where they were able to contact other dogs, as described below.

3.2.1. Temporal categorization of contacts and GPS positions

To evaluate the time component of contacts, data was organized in different time periods according to when the contacts and GPS fixes were recorded. It was possible to orchestrate the division of the day into 12 periods, using the timestamp information. Accordingly, the first period would start 1h before sunrise, and finish 1h afterwards, comprising an approximation of the twilight. This was followed by the remaining periods of 2 hours each, resulting in 12 periods over a 24h-day (Table 3). For accuracy purposes, the sunrise and sunset times from the specific dates and locations were used to build time categorizations according to the above-mentioned method for each country separately ("In-The-Sky.org" by Dominic Ford).

Table 3 - Time periods and correspondent time window assorted per study location, adjusted to the local time zones.

Time Period	**Soroti**	**Habi**	**Poptún**
A	05:53 – 07:54 Sunrise: 06:64	05:02 – 07:03 Sunrise: 06:03	04:21 – 06:22 Sunrise: 05:22
B	07:53 – 09:54	07:02 – 09:03	06:21 – 08:22
C	09:53 – 11:54	09:02 – 11:03	08:21 – 10:22
D	11:53 – 13:54	11:02 – 13:03	10:21 – 12:22
E	13:53 – 15:54	13:02 – 15:03	12:21 – 14:22
F	15:53 – 17:54	15:02 – 17:03	14:21 – 16:22
G	17:53 – 19:54 Sunset: 18:58	17:02 – 19:03 Sunset: 17:48	16:21 – 18:22
H	19:53 – 21:54	19:02 – 21:03	18:21 – 20:22 Sunset: 18:24
I	21:53 – 23:54	21:02 – 23:03	20:21 – 22:22
J	23:53 – 01:54	23:02 – 01:03	22:21 – 00:22
K	01:53 – 03:54	01:02 – 03:03	00:21 – 02:22
L	03:53 – 05:54	03:02 – 05:03	02:21 – 04:22

We aimed to include weekdays and weekend in all three study locations for the entire data collection period. However, due to short battery duration, data collection during a full week was not possible (Table 4). All days of data collection period were included in the analysis, despite incompleteness of the last days' data due to the uncollaring process.

Table 4 – Date and weekday of each study day per study location.

	Soroti	**Habi**	**Poptún**
Wednesday	Day I 23.01.2019	-	-
Thursday	Day II 24.01.2019	-	Day I 31.05.2018
Friday	Day III 25.01.2019	Day I 27.07.2018	Day II 01.06.2018
Saturday	Day IV 26.01.2019	Day II 28.07.2018	Day III 02.06.2018
Sunday	Day V 27.01.2019	Day III 29.07.2018	Day IV 03.06.2018
Monday	Day VI 28.01.2019	Day IV 30.07.2018	Day V 04.06.2018
Tuesday	-	Day V 01.08.2018	Day VI 05.06.2018

3.2.2. Spatial analysis

The geolocated contacts were mapped for each dog for the full data collection period and separated for each 2-hours' time period and study day, respectively. For that purpose, a grid of hexagons was created; each of them assigned a unique ID. The extent of the grid layer in each location was chosen so that it contained all the GPS fixes of all dogs in this location, for all time periods. However, as the grid application often took disproportionate dimensions due to certain GPS outliers, perhaps due to undocumented human displacement or singular dog off tracking, it was decided that only cells with at least two GPS fixes would be used to define the extent of the grid. Grid cells with less than two GPS fixes at the periphery of the area were ignored and the GPS fixed removed from the further analysis.

To characterize each of the grid cells concerning the number of contacts and GPS fixes, and consequently to identify hotspots of contacts, the Kernel Density Estimate (KDE) of the *Sfhotspot* package was used (Ashby M. 2023). The function *hotspot_KDE* calculated the KDE for the density of contacts and GPS points, whereas the *hotspot_dual_KDE* calculated the Contact:GPS density ratio for each grid cell. This ratio is generated by calculating the number of contacts over the number of GPS fixes in each cell, and thus it is a measurement of contact hotspots. Bandwidth for the kernel was determined automatically, a feature of the package's functions, by using the 'rule-of-thumb' for choosing the bandwidth of a Gaussian kernel density estimator. The package uses the function “bw.nrd”, Scott’s variation of the Silverman’s ‘rule-of-thumb’, which uses a factor of 1.06 (Scott 1992).

The spatial distribution of the locations with high KDE ratio values was explored visually. A boxplot panel graph was created to compare grid cell IDs found to have recurrently higher ratio values during different days of recording. Subsequently, using the package *ggplot2* (Wickham 2016), we overlaid the grid with their respective contacts and GPS KDE, and ratio values of these two KDEs, onto an OpenStreetsMap map (OpenStreetMap 2022). Finally, aiming at highlighting the cells that recurrently had a high ratio (indicating that there is a higher kernel density of contacts compared to the density of the GPS fixes KDE); the polygons with ratios higher than two were marked for each time period investigated. This enabled the identification of cells with more contacts than GPS points, classified as contact hotspots.

We also consulted the local team members to identify structures underlying the hotspot locations. The local team then visited the locations or reviewed registries from the time of data collected and indicated the city structures located at the positions of the identified hotspots. In order to complement these, Google maps was consulted to identify the sites of the hotspot and check for overlaps with structures.

3.2.3. Temporal analysis

For each location separately, a generalized linear model (glm) was used to assess whether the Contact:GPS density ratio was influenced by Time Period (the 12 time periods mentioned previously) and Study Day (the weekday of data collection), to identify periods where dogs were more often in contact with each other. The outcome variable of the model was the Contact:GPS density ratio, whereas the independent variables were Study Day and Time Period. Due to a left skew in the ratio distribution, it was necessary to transform the outcome variable into the natural log, as glm required a Gaussian distribution (Equation 1).

$$glm(log(ratio) \sim Time\_Category + Day,\ family = "Gaussian"). \qquad (1)$$

For the reference levels, Saturday, and Time Period D, were chosen, as it represented the first day of the weekend, with complete data recorded in all three locations, and the middle of the daytime, respectively.

4. Results

After data cleaning and merging of the two datasets (GPS data and contact data), 193 FRD across the three locations, Soroti, Habi, and Poptún, were included in the study. The study population comprised 61 dogs from Soroti, 66 dogs from Habi, and 66 dogs from Poptún.

4.1. Spatial analysis of contact hotspots

Our observations indicate a robust dispersion of GPS fixes across the study locations, displaying a lack of discernible barriers or constraints to dogs' roaming. The extent of coverage was most extensive in Poptún compared to the other locations (Figure 1a, c, e).

Regarding the spatial arrangement of geolocated contacts (Figure 1b, d, f), diverse patterns emerge across the distinct locations. Soroti show a less clustered distribution, standing in contrast to the more aggregated dispositions evident in Habi and Poptún.

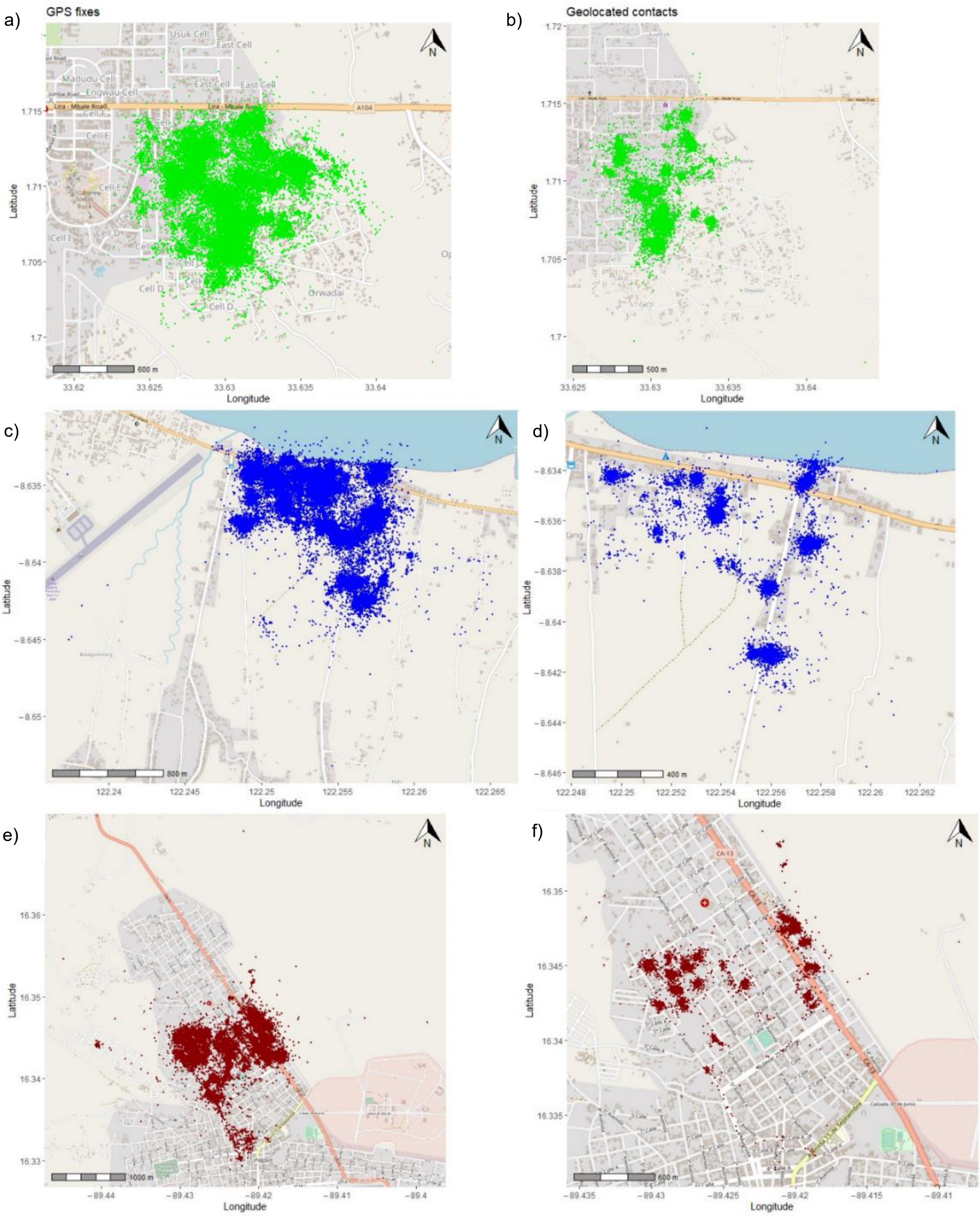


Figure 1 - **Map representations of: a) Soroti's GPS fix and b) geolocated contacts' disposition, c) Habi's GPS fixes and d) geolocated contacts' disposition, e) Poptún's GPS fixes and f) geolocated contacts' disposition**

After smoothing the spatial GPS and geolocated contact data from Figure 1 using the KDE functions for each Study Day and Time Period separately, it was again observed that the density distribution was not random (Figure 2).

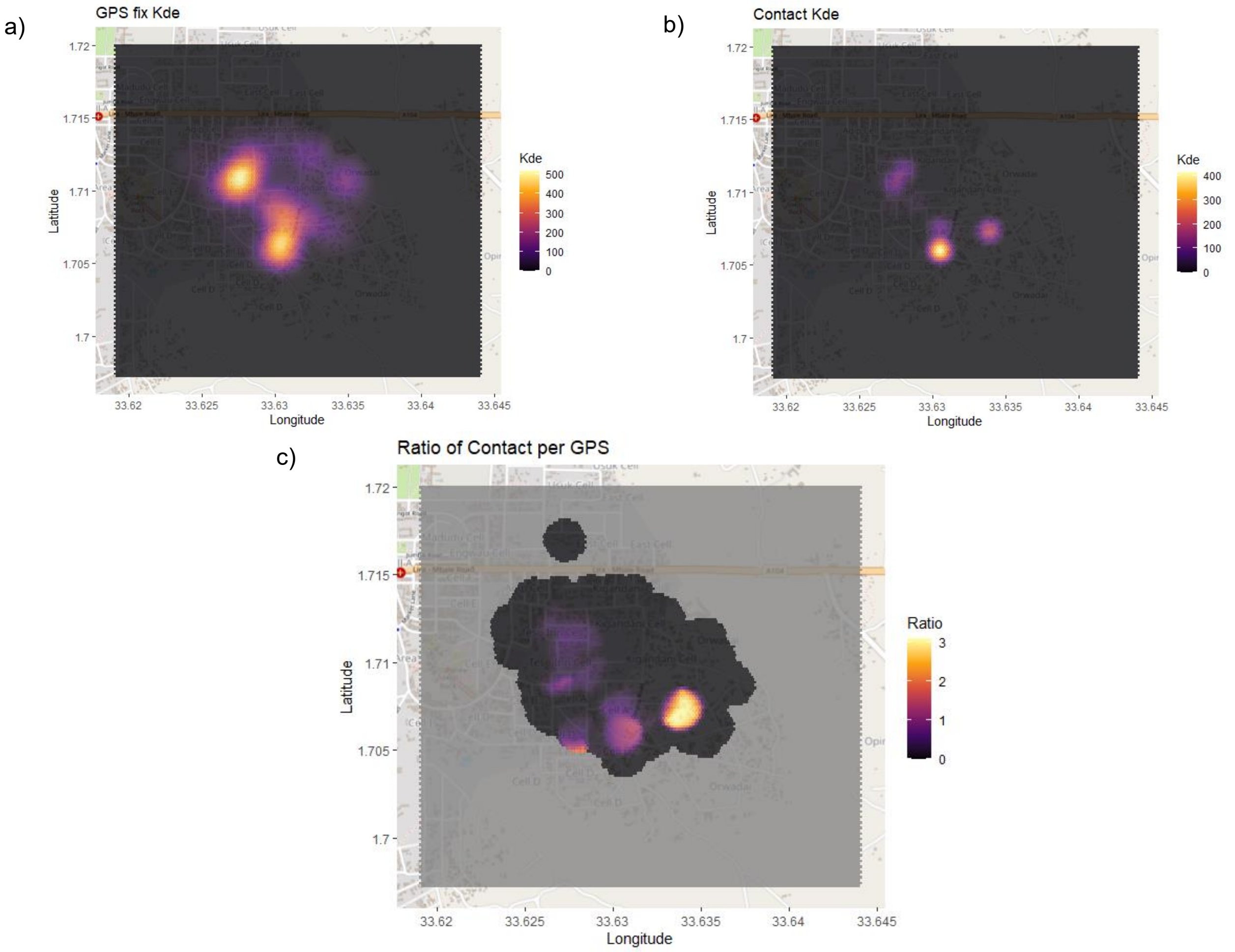


Figure 2 – a) KDE value distribution for the GPS fixes, b) KDE value distribution for the geolocated contacts, c) Contact:GPS density ratio, Saturday, time period I, Soroti.

When taking into consideration the higher KDE value, it was possible to identify areas where the dogs were often present (Figure 2a), and where dogs often contacted (Figure 2b). When the dogs' contact density was corrected for its GPS density, and the ratio value attained, new areas were identified as hotspots of high-contact and low-GPS-fix density (Figure 2c).

The same pattern was observed in the three locations and for the great majority of the time periods and study days (Annex repository). For the GPS fix (Figure 2a) and contact KDE (Figure 2b), the black colour throughout the study area represents a value of 0 KDE, as there was no data recorded in those sections. For the ratio (Figure 2c), the black colour represents a value of 0, whereas the light grey colour signifies lack of value, a not applicable (NA), given it was not possible to calculate the ratio in the absence of GPS fixes and contacts.

The distribution of hotspots across different geographical areas varied depending on the day and time period (Figure 3).

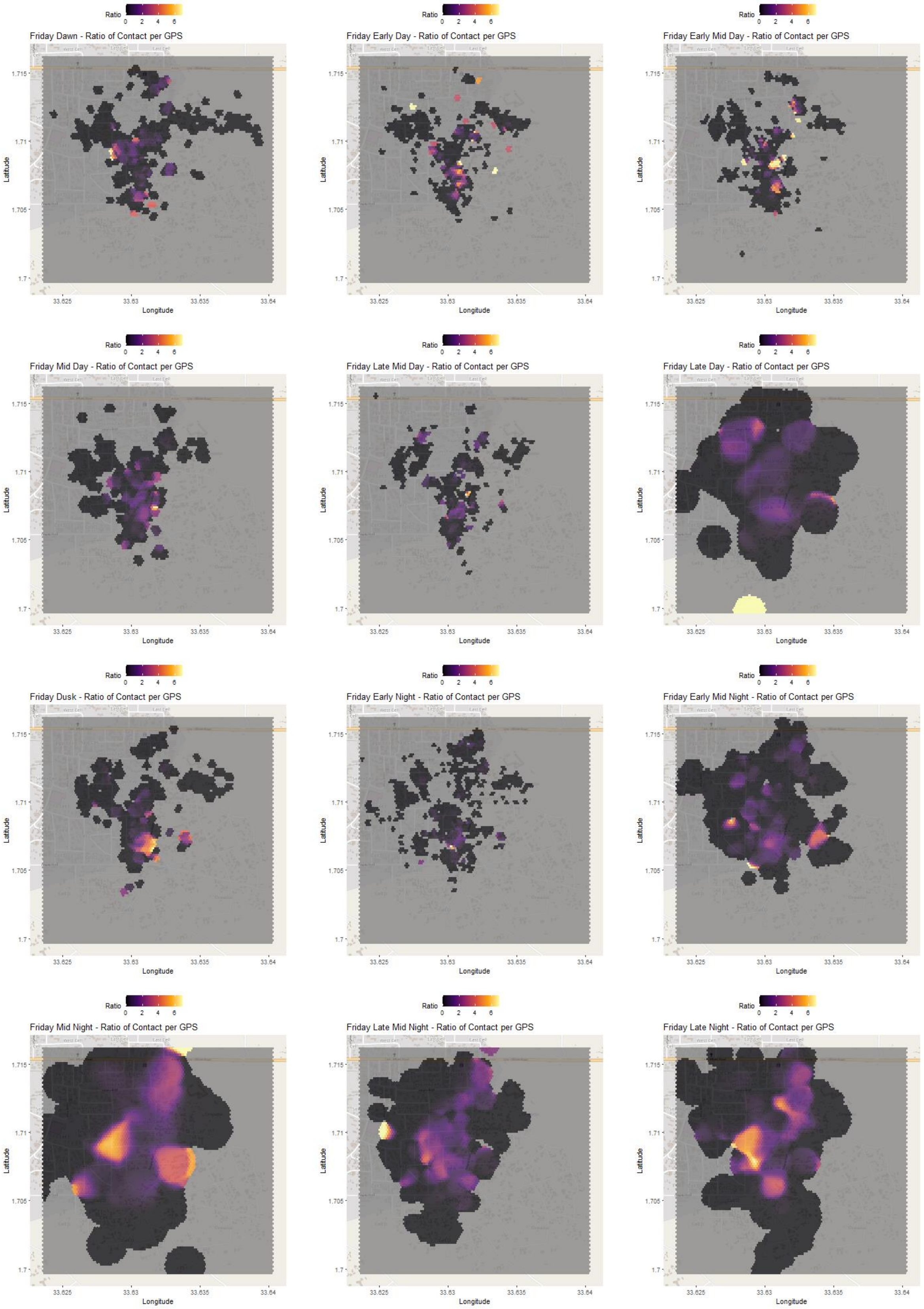


Figure 3 – Contact:GPS density ratio distribution over the 12 time periods of day III, Friday, in Soroti.

A dynamic and fluid pattern emerges when we examine the data chronologically, revealing the ever-changing locations of dogs and, subsequently, the shifting nature of hotspots. This observation underscores the variability in dog behaviour and contact patterns over time. At dawn, the first time period in Figure 3, it is possible to note that the hotspots do not carry onto the next time period, early day, but they also do not entirely disappear, retaining medium values of ratio. Similarly, the hotspots from the second time period do not all disappear or change, hinting at a repeating pattern in the locations of these hotspots. At varying time periods, the grid displayed a greater spatial spread of KDE, showing a larger area coverage. This led to greater dispersion of the dog's contacts in the city, resulting in larger areas of both high and low ratio, making the hotspot analysis less straightforward (Figure 3, 6th and 9th – 12th time periods). It is worthy of mention that the larger the spatial spread over the study area (larger grid necessary), the heavier the computational power/time needed for data processing.

The finding of spatial overlapping contact hotspots between time periods is also confirmed by the fact that clear clusters were detected when summarizing the hotspots over the entire study duration.

In Figure 4, the average of the ratio values over the time periods for each cell across all the study duration was outlined, revealing the formation of clustered hotspots. The spatial pattern of these hotspots varies between study locations. Soroti exhibits a more dispersed arrangement with minimal aggregation, whereas in Habi and Poptún, the clustering of hotspots is pronounced (Figure 4). Moreover, when the ratio values are averaged for each cell per study day, it is possible to observe that the ratio value is consistently higher in some cells, across the study-days (Annex 1, 2 and 3).

Upon consultation with the local team, it was found that the highlighted cells in Soroti overlapped schools and their vicinities. Contrastingly, in Habi, the hotspots overlapped an empty patch of shaded trees, a comfortable spot for dogs from the Habi neighbourhoods, but not exclusively according to local empirical evidence, to hang and lay around. In Poptún, the hotspots overlapped with a residential block and a commercial block containing a hotel, gift shop, gas station, food restaurants and a veterinary clinic.

Figure 4 - a) Soroti's cells with a consistently higher Contact:GPS density ratio throughout the study, school infrastructure outlined (blue). b) Habi's cells, shaded patch of vegetation outlined (green), hotel and shops (red). c) Poptún's cells, residential block (black), schools (blue), hotel/food-court/shops (red), veterinary clinic (yellow), outside urban planning (orange).

4.2. Temporal analysis of the contact hotspots

Each study location shows heterogeneous distribution of the Contact:GPS density ratio across and within the study days. Therefore, the ratio values exhibited the bellow-described dependencies on the study days and time periods.

In Soroti, during the daytime hours, the dogs' Contact:GPS density ratio is seemingly lower, whereas during the night it is higher (Figure 5).

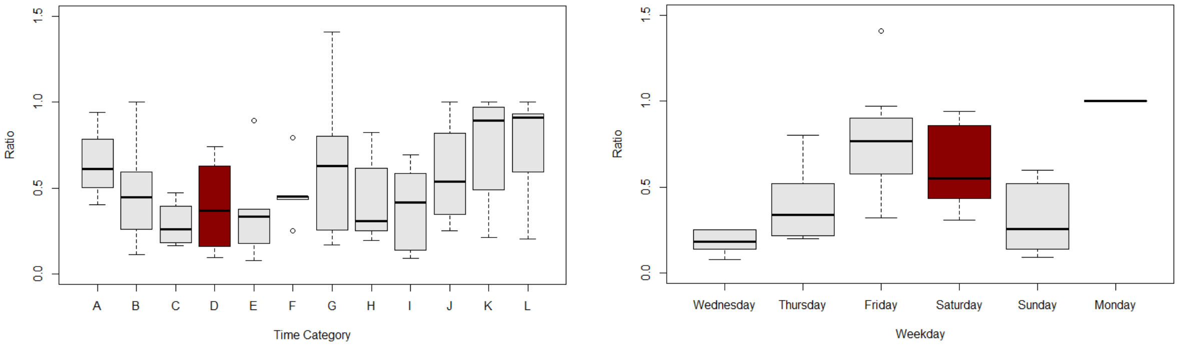
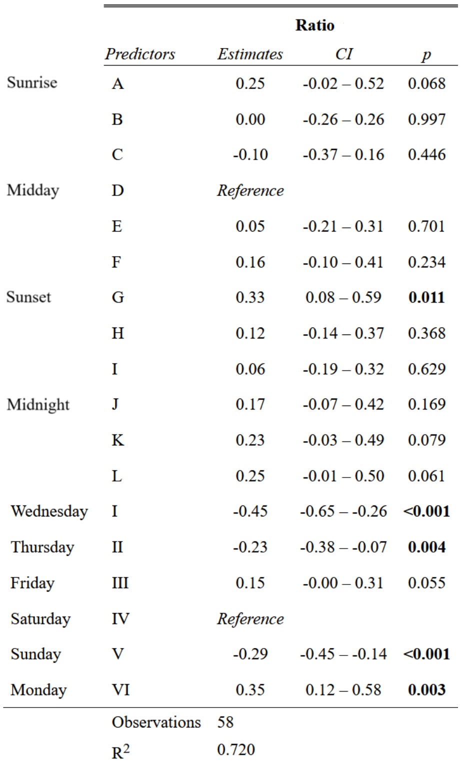


Figure 5 - Boxplot of the Contact:GPS density ratio distribution per time period and study day in Soroti, reference factors for the general linear model coloured red.

Additionally, a dog's contact ratio tends to increase as the weekend draws closer, peaking on Friday and reducing slightly onto Saturday and more intensely, Sunday and Monday. Concordantly, the time period G (dusk), is significantly more likely to have a higher ratio than time period D (Table 5).

Table 5 – Linear regression model results for Soroti



		Ratio		
	Predictors	*Estimates*	*CI*	*p*
Sunrise	A	0.25	-0.02 – 0.52	0.068
	B	0.00	-0.26 – 0.26	0.997
	C	-0.10	-0.37 – 0.16	0.446
Midday	D	*Reference*		
	E	0.05	-0.21 – 0.31	0.701
	F	0.16	-0.10 – 0.41	0.234
Sunset	G	0.33	0.08 – 0.59	**0.011**
	H	0.12	-0.14 – 0.37	0.368
	I	0.06	-0.19 – 0.32	0.629
Midnight	J	0.17	-0.07 – 0.42	0.169
	K	0.23	-0.03 – 0.49	0.079
	L	0.25	-0.01 – 0.50	0.061
Wednesday	I	-0.45	-0.65 – -0.26	**<0.001**
Thursday	II	-0.23	-0.38 – -0.07	**0.004**
Friday	III	0.15	-0.00 – 0.31	0.055
Saturday	IV	*Reference*		
Sunday	V	-0.29	-0.45 – -0.14	**<0.001**
Monday	VI	0.35	0.12 – 0.58	**0.003**
	Observations	58		
	R^2	0.720		

The linear regression model found an unevenly distributed ratio by day. Days I, II, and V, (Wednesday, Thursday, and Sunday, respectively) are significantly more likely to have a lower ratio than day IV (Saturday), whilst day VI (Monday), is more likely to have a higher ratio. Noteworthy, even though statistical significance was not attained (p=0.055), Friday is more likely to have a higher ratio than Saturday, signifying the weekly peak in the dog's Contact:GPS density ratio in Soroti.

In Habi, the ratio increases from dawn (A) until midday (D), exhibiting a drop in the consecutive time period (E), and raising back up again while the day progresses. The ratio values then remain stable, until early night (I), with a decrease afterwards (Figure 6).

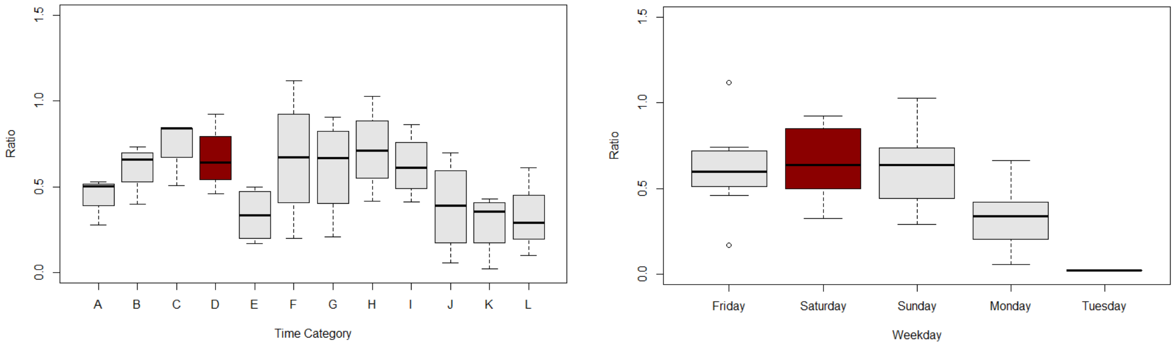


Figure 6 - Boxplot of the Contact:GPS density ratio distribution per time period and study day in Habi, reference factors for the general linear model coloured red.

Additionally, a decreasing trend is notable in the weekdays as Friday, Saturday and Sunday hold higher values than Monday and Tuesday, peaking on Saturday and plunging on Tuesday. Retaining only the statistically significant values, time periods E, J, and L (periods early afternoon, midnight and before dawn), are significantly less likely to have a higher ratio, than time period D, whereas day IV and V (Monday and Tuesday) are significantly less likely to exhibit a higher ratio than day II (Saturday) (Table 6).

Table 6 - Linear regression model results for Habi.

		Ratio		
	Predictors	*Estimates*	*CI*	*p*
Sunrise	A	-0.21	-0.49 – 0.06	0.126
	B	-0.05	-0.33 – 0.22	0.701
	C	0.08	-0.19 – 0.35	0.569
Midday	D	*Reference*		
	E	-0.33	-0.58 – -0.08	**0.010**
	F	-0.00	-0.25 – 0.25	0.993
Sunset	G	-0.05	-0.30 – 0.20	0.681
	H	0.05	-0.20 – 0.30	0.699
	I	-0.04	-0.30 – 0.21	0.734
Midnight	J	-0.28	-0.53 – -0.03	**0.028**
	K	-0.27	-0.54 – 0.00	0.053
	L	-0.32	-0.59 – -0.04	**0.023**
Friday	I	-0.06	-0.24 – 0.12	0.512
Saturday	II	*Reference*		
Sunday	III	-0.04	-0.19 – 0.10	0.553
Monday	IV	-0.33	-0.48 – -0.19	**<0.001**
Tuesday	V	-0.48	-0.90 – -0.07	**0.024**
	Observations	44		
	R^2	0.684		

Lastly, in Poptún, a significant dispersion of ratio values was observed throughout the study, resulting in a wide confidence interval of the ratio values. This pattern is with the exception of midday (D) and early afternoon (E), which exhibit narrower ranges within each respective time-period. Consequently, due to the absence of consistent trends and the lack of statistical significance across time periods, no clear increase or decrease of the values can be identified. Looking at the weekdays, a decreasing trend is noted from Thursday on, reducing abruptly on Sunday and Monday, and a spike on Tuesday (Figure 7).

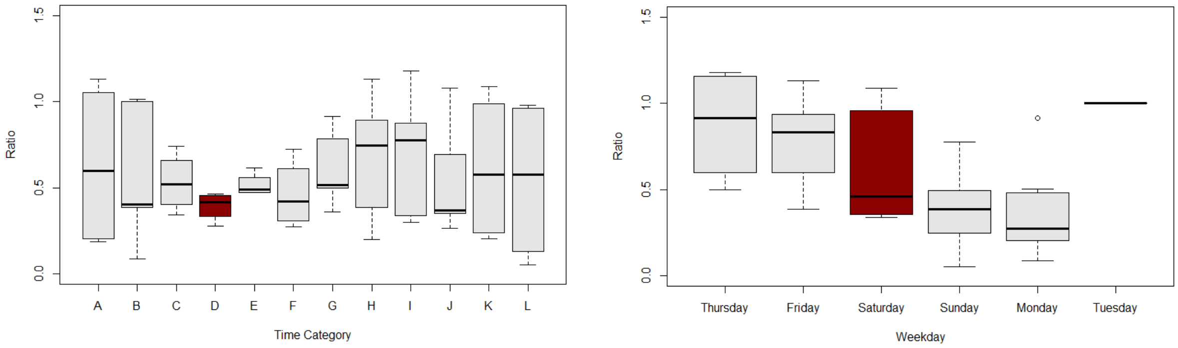


Figure 7 - Boxplot of the Contact:GPS density ratio distribution per time period and study day in Poptún, reference factors for the general linear model coloured red.

Considering only the statistically significant ones, day V (Monday) was found to be significantly less likely to have a higher ratio than day II (Saturday) (Table 7).

Table 7 - Linear regression model results, Poptún.

		Ratio		
	Predictors	*Estimates*	*CI*	*p*
Sunrise	A	0.23	-0.15 – 0.62	0.233
	B	0.08	-0.31 – 0.47	0.687
	C	0.14	-0.25 – 0.52	0.484
Midday	D	*Reference*		
	E	0.12	-0.26 – 0.51	0.534
	F	0.07	-0.32 – 0.45	0.736
	G	0.16	-0.21 – 0.53	0.397
Sunset	H	0.22	-0.15 – 0.59	0.253
	I	0.24	-0.13 – 0.61	0.205
Midnight	J	0.10	-0.27 – 0.47	0.609
	K	0.22	-0.17 – 0.61	0.267
	L	0.15	-0.23 – 0.54	0.438
Thursday	I	0.22	-0.11 – 0.55	0.197
Friday	II	0.16	-0.07 – 0.38	0.170
Saturday	III	*Reference*		
Sunday	IV	-0.22	-0.44 – 0.00	0.055
Monday	V	-0.27	-0.50 – -0.05	**0.016**
Tuesday	VI	0.44	-0.18 – 1.07	0.165
	Observations	53		
	R^2	0.451		

5. Discussion

Despite the enduring relationship between dogs and humans, questions still remain about how dogs interact with each other on ecosystems, particularly in urban areas that have experienced rapid human population growth and where dogs, consequently, prospered and developed specific dynamics, to enable them to persist in a human territory.

Given the ecology of rabies, One Health approaches are being pushed forward, representing perhaps the biggest chance humanity has ever held of eradicating the disease. The United Against Rabies Forum (UAR) is currently promoting cross-sector collaboration among stakeholders, motivating both political and popular engagement in control campaigns (Tidman et al. 2022).

Population conducts regarding the catering of FRD, should be tackled through awareness campaigns, calling for sociology professionals, whilst disease control at the source calls for veterinary care, finally improving public health and wellbeing of the populations. Concordantly, in this study, we delved into the intricate dynamics of FRD in three distinct locations, Soroti in Uganda, Habi in Indonesia, and Poptún in Guatemala, where we collared FRD and analysed spatial data through kernel density estimates. The analysis of dog movement patterns and contact rates presented in the results section shed light onto several crucial aspects.

5.1 Distribution of hotspot

Primarily, it should be noted that the dogs' spatial dispersion, or general map coverage, was not constant throughout the different days. Hinting that, although location-specific, dogs seem to roam at specific time periods of the day, likely in concordance with peaks in their activity. However, in order to draw conclusive interpretations, it would be necessary to look into each dog individually, as it has been shown that some dogs roam, and contact, far more extensively than others (Wilson-Aggarwal et al. 2019). A dog whose contact and roam patterns far outweighs their peers is sure to mask the other's distribution using the present methodology.

Next, barriers to the dog's roam could not be identified, as the spatial dispersion was marked across all locations. Especially so, when taking into account the above-presumed differences in FRD activity; considering the smallest spatial dispersion as a FRD resting baseline, it is then possible to note that an extensive range of movement is present, being such differences less pronounced in Habi. However, the above-mentioned dog heterogenicity applies here as well. Although current methodology and findings can only suggest it, a seemingly barrierless roam of domestic dogs is likely to increase spatial dispersion of the human dog interface and have significant implications for rabies transmission dynamics. Understanding what enables such free movement, leading to extensive contacts could help shape targeted intervention strategies, particularly awareness on community practices (de Melo et al. 2023).

5.2 Temporal analysis

Our analysis revealed intriguing day and time effects on dog contact ratio. For example, in Soroti, the ratio was lower during daytime hours but increased during the night, hinting at a nocturnal contact pattern (Figure 5), opposing to the activity bimodal standard reported by Beck (1975). Contrary to Soroti, in Habi, while high contact rates may not directly imply high dog activity; they certainly suggest an active dog presence. This also implies that even though dogs have a bimodal biological clock, the potential for disease transmission remains.

Another intriguing finding was that weekends, especially Fridays, showed the highest contact ratios across locations. Underlining a weekly pattern of contact rates per location, that steadily reduced once it peaked on Friday, possibly associated with communal gatherings and activities in the locations. Leading to events that could attract FRD via littering and scavenging or attention seeking. As these variations likely stem from both dog behaviour and ownership practices, given activity patterns are also dependent on the owners' activity (Griss et al. 2021), it is possible to hypothesise that the increase in human spatial dispersion during the weekend, positively influenced FRD roaming. Understanding these patterns, can inform policymakers about the best timing of vaccination campaigns and the aim of awareness initiatives (WHO 2018). For example, identifying disease transmission hotspots and investigating their correlation with human routines, such as free-roaming dogs gathering around schools during specific hours, students' commuting patterns, or weekend communal events, becomes critical for policymakers. This knowledge allows for targeted focus on specific demographic groups in human populations, influencing their awareness and practices. It also allows directing vaccination, or monitoring efforts, toward contact hotspots that, due to their proximity to key infrastructure or wildlife habitats, pose a high risk of introducing diseases into urban areas or causing spillover events. These insights are invaluable for optimizing resource allocation and improving the overall effectiveness of rabies control programs.

In Guatemala, owners are often accompanied by their dogs, whereas in Indonesia and Uganda, this practice is not widespread (Warembourg, Wera, et al. 2021). Such fact could explain the differences in the linear regression modelling, where the Guatemalan scenario presents a widespread distribution of ratios within each time period, lacking statistically significant differences. Therefore, it is possible to suggest the FRD heterogenicity is not restricted to the intrinsic nature of the dog, but also to the attitudes and practices of their owners. Reinforcing this way that knowing the type of dog owner is crucial for rabies control.

5.3 Spatiotemporal Analysis

Further, when taking the temporal aspect into the equation, it was possible to perceive that the spatial-temporal patterns of contacts between dogs in the three locations investigated, was very distinct. This finding offers insights into the complex dynamics of dog roaming behaviour. The patterns might be influenced by a combination of factors, including human activities, environmental conditions, and local dog populations' demographics (Vanak and Gompper 2009; Bhattacharjee and Bhadra 2020; Cunha Silva et al. 2022). For instance, the higher concentration of contacts in specific areas during certain time periods might be linked to routines such as communal activities or feeding times (Griss et al. 2021; de Melo et al. 2023). Our findings emphasize the need for a more detailed evaluation of these factors to better understand and predict dog contact patterns. Through the information on the time periods and locations where FRD contact is most frequent, activity and planning of institutional intervention can be regulated and optimized. Interventions that directly target dogs, such as enumeration campaigns or trap-neuter-release programs, are more likely to succeed if it is at a time dogs are the most active, and in areas of FRD high density.

On the other hand, when monitoring FRD's ecology, one should consider both high GPS fix density and high contact density zones. Should policy makers assess only areas with high GPS fix density, high contact but low GPS fix density areas are likely to be overlooked and may pose a risk for silent transmission. Logically, areas with high FRD density are prone to facilitate contacts between dogs, and it was noticed that areas with the high GPS fix KDE held a high contact density. However, when considering the ratio, where the contact density frequency is adjusted for the density of GPS fixes, new locations of possible interest were found (Figure 2). These locations represent areas with high intensity of contacts, regardless of the GPS fix density. Such patterns, when spatial or temporal consistency is noted, excluding this way random dog displacement in space, may highlight targets shared by the FRD, a common purpose in that roam event. Then it may be possible to identify locations or infrastructures FRD are drawn to. The significance of these hotspots lies in their potential role for rabies transmission and control.

Moreover, considering high GPS fix, or habitat selection, to define FRD high-density locations helps delineating areas of high risk for humans in endemic countries and areas of interest for enumeration, capture-recapture activities, or ORV campaigns (Cunha Silva et al. 2022). Whereas locations with a high Contact:GPS density ratio provide valuable insights for disease transmission monitoring. This information enables the development of models describing the likelihood of transmission at a given location and time, and, in the event of an outbreak, allows for the implementation of precautionary measures. These measures are essential for breaking the infectious chain, and posteriorly identifying the origin, in the event of a spillover. Investigating the characteristics of these hotspot areas could provide crucial insights into disease control strategies (Wright et al. 2021). Intervention-wise, and assuming contact hotspot areas are relevant for disease transmission, these could also serve as focal points for targeted vaccination campaigns (Wieten et al. 2013) or high-success rate drop sites for ORV (Bobe et al. 2023; Leelahapongsathon et al. 2023).

The observed spatial-temporal consistency in hotspot patterns across study days and time periods is a noteworthy finding. It was described that FRD GPS-fix and contact density would be consistently higher in specific locations, throughout the study periods. Additionally, it was observed that the hotspots often spanned more than a single day (Annex 1, 2 and 3). This consistency suggests that certain dog behaviour patterns, likely influenced by environmental and human factors, are stable over time. For disease control, understanding these regular patterns is advantageous as it allows for the design of interventions aligned with these patterns, optimizing resource allocation, and gaining efficiency. For instance, as FRD contact hotspots play a crucial role in infectious disease dynamics, policymakers can establish surveillance protocols on these locations. Such could involve passive surveillance by observing changes in the behaviour of FRD or in an outbreak suspicion due to an increase in the number of human or dog cases, active surveillance may be implemented to trace back, contain the afflicted, and eliminate the source of the spillover event. Moreover, considering the heterogeneity of FRD, and assuming those that roam the most are likely to be present in said hotspots, it would be beneficial to establish a ring vaccination strategy in these potential transmission hotspots. This can be achieved through campaigns involving the capture and vaccination of dogs or by deploying ORV baits. Constituting then an additional barrier to FRD-borne zoonotic disease introduction in urban settings.

5.4 Cross-location analysis

Comparing the distribution of hotspots in different study locations highlights the role of local factors in shaping dog behaviour. For instance, Soroti exhibited a far more dispersed arrangement of hotspots. Whereas Habi and Poptún displayed a more clustered arrangement (Figure 4), suggesting these may have been subject to distinct realities. Concordantly, it hints that the attitudes towards FRD, practices of dog owners, demographics of the FRD population, and urban settings have effect on their distribution throughout their habitats (Bhattacharjee and Bhadra 2020; Warembourg, Wera, et al. 2021; Cunha Silva et al. 2022). On the same note, and given piscatorial practices in Habi, this also supports the existence of highlighted cells in the coast and pier (Figure 4b), where anglers are accompanied by their dogs. It is also possible to suggest that a less demarked hotspot disposition, such as seen in Soroti, or a greater spread of highlighted cells, i.e., grid cells with persistently higher Contact:GPS ratio, may point out lack of residue management, considering FRD can survive solely on human waste products (Beck 1975). Tailoring governmental intervention efforts in waste management may help reducing the extent to which these dogs roam (Wright et al. 2021). Or, on the other hand, increase it, due to the foraging nature of FRD, which could cause further conflicts with both humans and wildlife, increasing the likelihood of a spillover, though deemed unlikely (Doherty et al. 2017; Nayeri et al. 2022).

The infrastructures overlapping with hotspots were quite diverse. In Soroti, the local team identified several schools in proximity or being at the centre of the hotspots. This underscores the risk of rabies transmission within the population of FRD, and the potential threat to children. Given the significant likelihood of conflicts between dogs and children, it becomes even more compelling to investigate the underlying causes, especially considering that children are the most affected demographic by dog-mediated rabies (Butcher and Keuster 2013). According to Beck and Jones (1985) children are keen on interacting with dogs, or vice-versa, dogs are attracted to them, prompting injuries by FRD (Beck and Jones 1985). While these contact hotspots may not necessarily be high-density areas for FRD, the fact that a disproportionate number of dog bites occur in children, raises the significance of these hotspots. This underscores the urgency in identifying why dogs tend to cluster around schools, with the aim of either eliminating the source of the hotspot or raising awareness among children or school personnel.

Conversely, Habi exhibited a distinct cluster of hotspots, remarkably associated with a shaded vegetation area (Figure 4b). Cunha Silva et al. (2022) also found in the same location that “low vegetation” was a preferred location of FRD (Cunha Silva et al. 2022). This suggests a potential influence of climate on the behaviour of FRD, as dogs are inclined to conserve energy when weather conditions are unfavourable (Beck 1975). However, it is plausible that this clustering could be attributed to breeding behaviour occurring within the observed period (Dürr et al. 2017). Moreover, though less conspicuously, there were overlaps with a hotel and convenience shops (Figure 4b), which raises the hypothesis that waste management practices at these establishments or tourist feeding FRD might be contributing factors (Beck 1975; Hillström et al. 2022).

Finally, Poptún presented the most miscellaneous case, perhaps due to the large nature of the urbanization, covering the largest area of this study, with hotspot clusters being found outside the city boundaries, near a residential block, schools, veterinary hospital, food-outlets, shop and hotels (Figure 4c). The findings in the latter, in line with those in Habi, may suggest that the attitudes and practices of tourists, including behaviours like feeding, could contribute, when on a large scale, to an increase in FRD populations. Consequently, this increase in numbers may elevate the risk of FRD-mediated diseases spreading to the local human populations (Hillström et al. 2022). The hotspots at the veterinary clinic may be due to, though unlikely, given the proximity to other infrastructure, and the consistency of the hotspots, that the team of the veterinary clinic feeds stray dogs, causing the observed hotspots. Such may prove to be a good opportunity for intervention, with the appliance of ORV, or its employment in capturing and neutering FRD, symbolising the potential the veterinary clinic can exert in the disease control in urban settings. If not for their practices, at least the proximity of the hotspot could motivate such action. Additionally, it was noted that hotspot clustering was occurring outside the city planning, on the forest (Figure 4c), and not too far from the biggest cluster of hotspots, already inside of the urbanization. These finding points out an increasing risk of spillover events, given FRD and wildlife are likely to cross paths, due to their foraging nature (Doherty et al. 2017; Nayeri et al. 2022). Finally, the household hotspot cluster may indicate community-feeding practices (de Melo et al. 2023).

Another important outcome of this study was that, despite the distinct characteristics of the study locations – with Soroti and Poptún being urban and Habi semi-urban – there was a remarkable consistency in the overlapping of infrastructure within the identified hotspots. In particular, the presence of schools overlapped with hotspots in Soroti and, to a lesser degree, in Poptún. Meanwhile, hotels and shops were commonly found in both Habi and Poptún. This suggests that, despite the differences between semi-urban and urban settings, the types of infrastructure that attract dogs remained steady across the locations. Furthermore, in both Habi and Poptún, there was clustering of contact hotspots beyond areas associated with human infrastructure, signalling a widespread risk of potential spillover events (Escobar et al. 2023).

5.5 Limitations and considerations

We acknowledge several limitations in our study. Firstly, weather and climate's disease determinants were not investigated, and they may be responsible for differences in temporal and spatial categorization of FRD (Warembourg, Wera, et al. 2021). The data analysed was gathered throughout time and in multiple locations (Table 4), leading to likely climacteric variation that could have influenced results. The data collection in Soroti and Habi took place during the dry season, whereas in Poptún, it coincided with the wet season.

Secondly, it is necessary to mention that not all dogs in these locations were collared, and thus, the current results represent only a subset of the FRD population. Therefore, the interpretation of results should be approached with the awareness that they represent a sample rather than a comprehensive depiction of the total FRD population. This limitation underscores the need for caution in generalizing findings and points towards the necessity of more extensive and inclusive studies to enhance the robustness of future research in this domain.

Further, it is worth noting that the data from the first and last days of the study locations was incomplete, as it did not cover the entire 24-hour cycle. This limitation, evident in Figure 5, 6, and 7, resulted from the time-intensive process of collaring and de-collaring all dogs. Despite this constraint, we opted to include these days in the study due to the substantial data available, including a significant number of dogs and GPS fixes. This decision was driven by the maintenance of statistical significance in our findings, as dogs would still exhibit high Contact:GPS fix ratios in the included time periods of the incomplete days if applicable, just lower dispersion of values, given the smaller sample size. We anticipated that days with less data might naturally present lower or higher ratios than they would realistically, making their comparison to complete study days challenging at the beginning or end of the data collection period. Therefore, when comparing the incomplete days, one must investigate the referenced time-periods and the number of FRD included in those days.

Lastly, this study utilized KDE methodology, which lacks statistical significance, acting as a mere indicator of potential locations of interest (Kalinic and Krisp. 2018). Additionally, faulty bandwidth definition may have led to incorrect transformations of the density plots, which may have contributed to i) under smoothing, if the value was too close to zero, leading to a combination of individual peaks; ii) or over smoothing, if the value was near the maximum value of the kernel, leading to a unimodal distribution. The first resulted in an oversensitive model where every peak becomes relevant, and the latter undermined every peak in data. Both resulted in loss of information and may rendered a KDE deceitful. Nonetheless, this study tried to cover for this limitation by observing the clustering of hotspots and by defining a general grid, for each location, including all the GPS fixes that acted as baseline for the KDE calculations. This methodology was then followed by the separation of the data according to the day and time, and for each instance, the bandwidth was calculated, assuring that proper values are used. Additionally, infrastructure overlap definition was done resorting to Google maps and local photographs, however, as lack for certainty regarding which infrastructure was present at the time of data collection arose, local teams were contacted, and their inputs integrated.

Nevertheless, building on the insights gained from this study, future research should consider the above-mentioned limitations, as they are avoidable in the design phase. It would be interesting to delve deeper into the factors influencing dog roaming behaviour: investigating the overlap with key locations; community activities and practices that likely influence the distribution of FRD; governmental intervention/expenditure towards the issue, by their own means, or through awareness; if waste management is done properly by stakeholders and officials; and socioeconomic factors since they could provide a more comprehensive understanding of dog movement patterns, in order to act on specific population demographics (Warembourg, Fournié, et al. 2021). Furthermore, extending this research to a wider range of geographic locations, would help validate the generalizability of our findings. Particularly, investigating different topographies, finding concordant infrastructures and demographics could further deepen the knowledge about FRD dynamics. Whether controlling contact hotspots would decrease urban transmission significantly to the point it becomes worth implementing i.e., through active/passive surveillance, hotspot cluster location monitoring for spill-over events, the creation of immunized high-contact FRD populations, raising awareness and the checking for correlation with the hotspot cluster patterns.

6. Conclusion

This study introduces a novel, minimally disruptive observational approach for revealing high-contact locations among free-roaming dogs (FRD), offering insights into the intricate spatial and temporal patterns of dog contacts in rabies-endemic regions. The research focused on Soroti (Uganda), Habi (Indonesia), and Poptún (Guatemala), employing data synthesis from GPS and contact datasets of 193 collared FRD. The following key conclusions emerge:

The analysis revealed a weekly pattern of contact rates, notably peaking on Fridays. This surge is suggested to be associated with communal gatherings and activities among people, potentially attracting FRD through littering, feeding, or attention-seeking.

The spatial-temporal consistency in hotspot patterns across study days and time periods underscores stable dog behaviour, influenced by environmental and human factors. Recognizing these regular patterns is advantageous for designing interventions aligned with these dynamics, thus optimizing resource allocation, and gaining efficiency.

Cross-location analysis highlighted the role of local factors and human infrastructure in shaping FRD distribution. Despite the differences between semi-urban and urban settings, there was remarkable consistency in the types of infrastructure within hotspots across locations.

This study contributes valuable insights to the broader goal of rabies eradication by 2030. Understanding FRD roaming behaviour is pivotal for effective rabies control. The spatial and temporal patterns uncovered emphasize the need for tailored strategies that account for the inherent heterogeneity in dog behaviour across diverse urban settings.

In conclusion, this research advances our understanding of the complex spatial and temporal dynamics of FRD contacts in rabies-endemic urban and semi-urban regions. The findings advocate for tailored control and surveillance strategies, acknowledging the inherent heterogeneity in dog behaviour across diverse settings. Recognizing these patterns and understanding local influences on FRD dynamics are pivotal steps toward optimizing disease control efforts and advancing the goal of rabies eradication by 2030.

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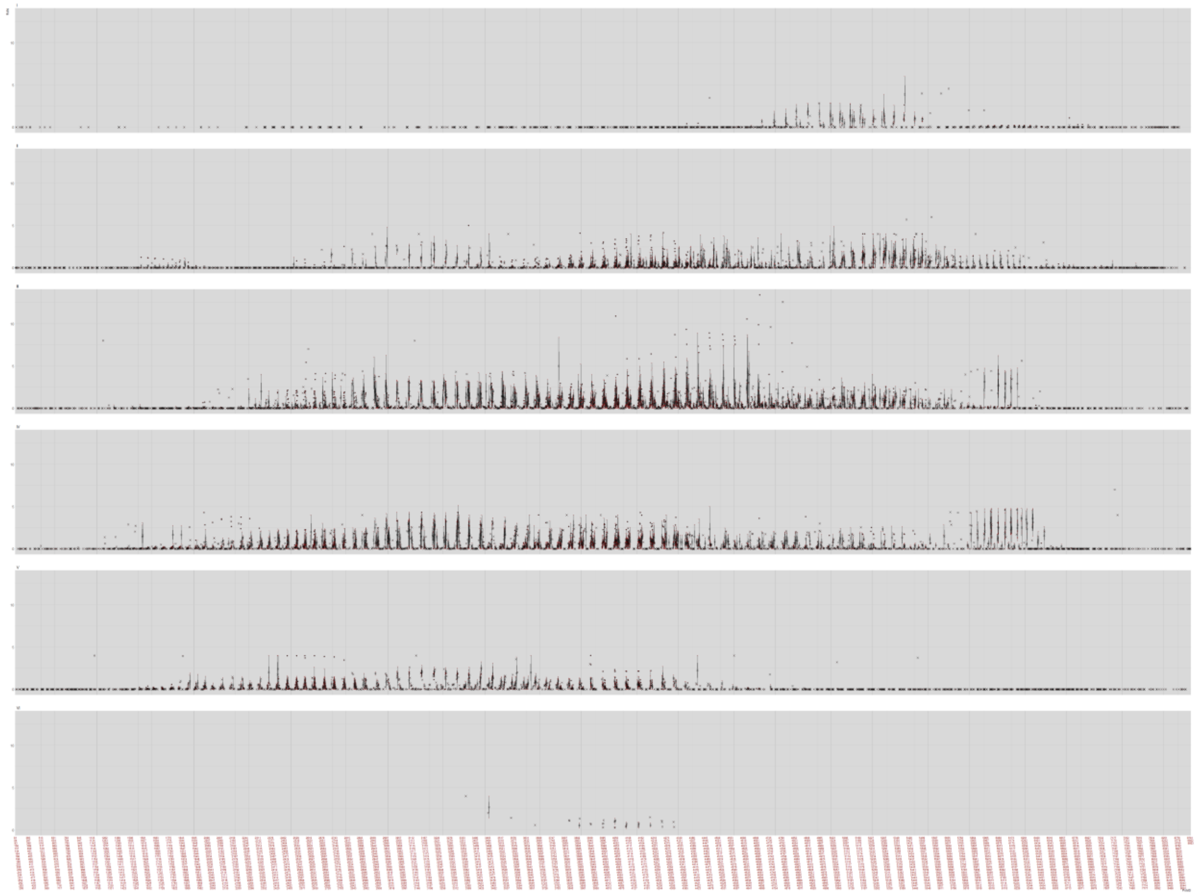
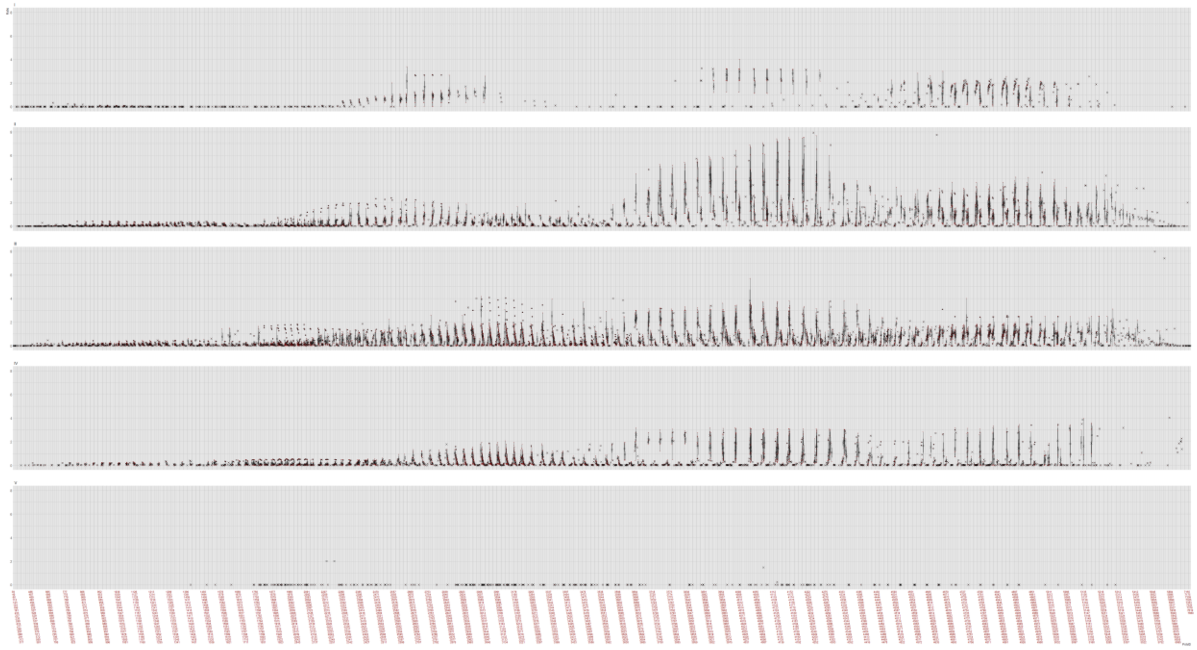
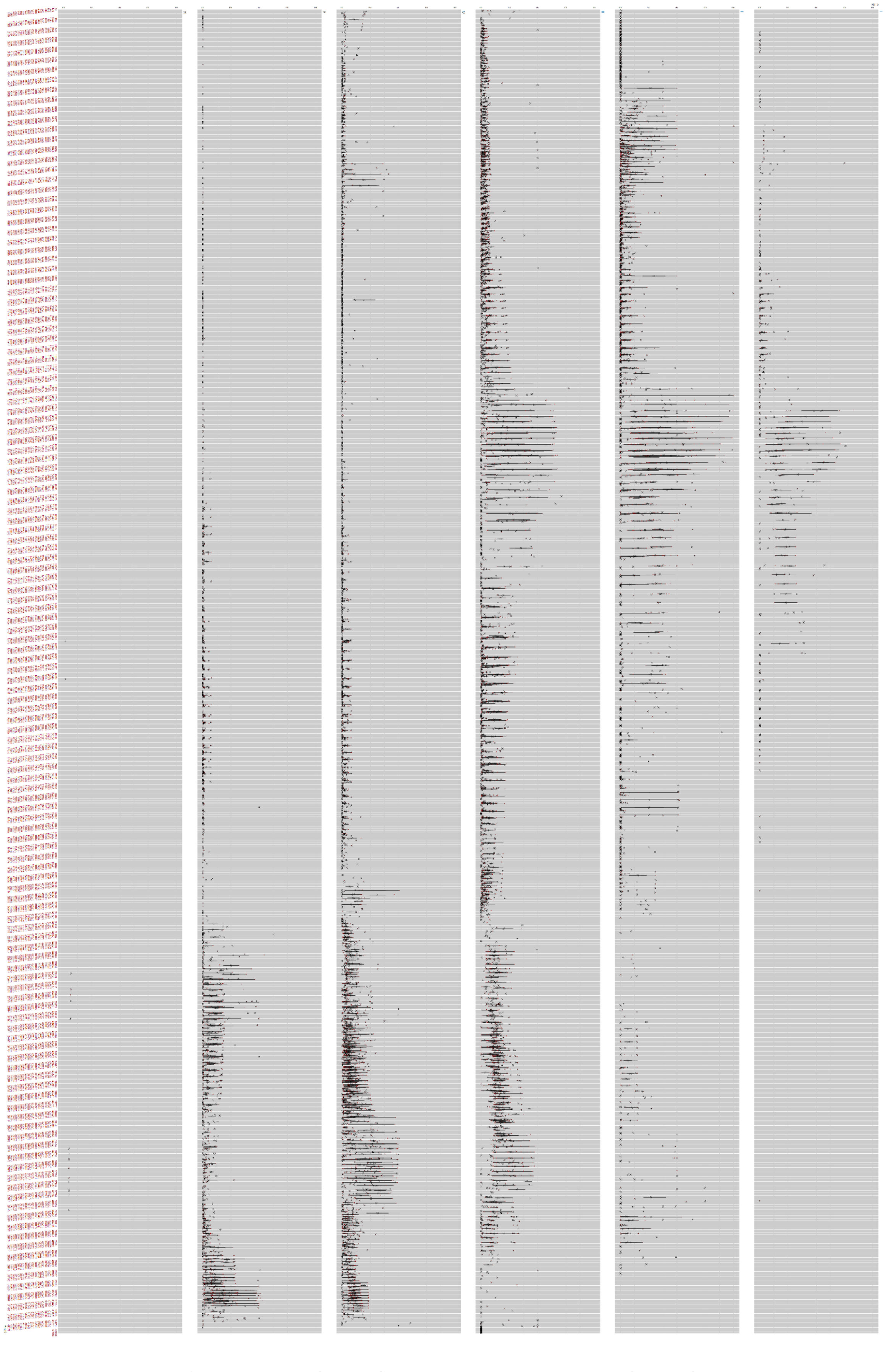
8. Annexes



Annex 1 - Boxplot of all the cells (x-axis) and the respective ratio (y-axis), throughout the study days in Soroti.



Annex 2 - Boxplot of all the cells (x-axis) and the respective ratio (y-axis), throughout the study days in Habi.



Annex 3 - Boxplot of all the cells (x-axis) and the respective ratio (y-axis), throughout the study days in Poptún.