Title: Impact of human-animal interactions in highly anthropized environments: An Urban One Health approach based on the example of the European hedgehog (*Erinaceus europaeus*).

State of the art and scientific questions

The emergence and the spread of human diseases linked to global change and the development of human activity become nowadays a major issue. For instance, the human population density is a good predictor of the emergence of infectious diseases. From a public health point of view, the urbanisation may have important consequences when pathogens circulating in rural environment adapt to urban environment and induce their emergence or re-emergence in urban areas (Hassel et al. 2017). Accordingly, the incidence of emerging pathogens has considerably increased the last 20 years, and, this increase may accelerate in a near future because of the global change and the increasing urbanisation (Hassel et al. 2017; Jones et al. 2008). The majority of human emerging pathogens comes from zoonotic reservoir (Jones et al. 2008), and the functioning and evolution of animal population are mainly affected by anthropic factors in our ecosystems. Consequently, such emerging diseases will continue to be a threat as long as the anthropic factors impacting animal populations hosting such pathogens have not been identified. In particular, some practices have recently emerged to attenuate the negative effects of global changes on biodiversity especially in urban environment where local authorities have promoted the development of green areas and wildlife care and rehabilitation centres. Indeed, such practices may help the installation of animal species in cities but may, in return, favour the emergence of zoonotic pathogens classically found in rural areas, in urban areas.

This is the case of the European hedgehog which is now found in green areas of several cities. It becomes important to know to what extent such species can constitute a "Trojan horse" for pathogenic microorganisms potentially transmissible to humans and the possible impact of these new green areas in terms of emerging infectious diseases and public health. In this context, our research team has recently identified the presence of Lyme diseases on ticks found on hedgehogs of "Montsouris Park" (Paris, 14th district). To answer these questions, an interdisciplinary team has been set up at the interface between ecology and veterinary sciences to propose this original PhD project. Clearly, such a project has public health issue and falls within the "Urban One Health" context.

PhD project objectives and hypotheses

The first objective of the project will be to evaluate the public health hazard for city dwellers related to the presence of the hedgehog in urban green areas, especially for people who come into contact with those animals. Our hypothesis (**hypothesis 1**) is that the development of green areas in city has promoted the implementation of hedgehogs which hosted several zoonotic pathogens that become present in town. Especially, we will focus on ticks (*Ixodes sp.*), the Lyme disease agent (*Borrelia burgdorferi s.l.*) and a ringworm agent (*Trichophyton erinacei*).

The second objective will be to examine how the trace metal exposure, a component of urban environment, may negatively impact the hedgehog immunocompetence, as lead is known to be immunotoxic for urban species (Chatelain et al. 2016). Such negative effects on hedgehog immunity may favour the spread of pathogens such as ticks, *Borrelia* and *Trichophyton erinacei* (Gasparini et al. 2014, Le Barzic et al. 2020; **hypothesis 2**).

The third objective will be to examine the effect of the duration of hospitalisation of hedgehog in wildlife centres on their health status. To favour animal welfare and care, the authorities have set up wildlife centres which enable for dwellers to bring distressed animals and to provide first aid. Once the animal has recovered, it is rehabilitated and released in suitable environments which may help to maintain population. The hedgehog is a popular animal and the number one mammal brought into wildlife centres. The effective duration of hospitalisation can greatly vary (from a few days to several months) among individuals. Such hospitalisation may increase the stress of hedgehogs, induce immunodepression and favour the reactivation of pathogens and hospital acquired infections (**hypothesis 3**).

Material

We will consider hedgehogs received at the wildlife centre of Maisons-Alfort Veterinary College (CHUV-FS; 950 admissions in 2021). We will establish a cohort of 500 hedgehogs, collected in

various places in Ile-de-France region, along an urbanisation gradient. We will make sure to recruit animals from both sex and age categories (juveniles *versus* adults), admitted through the year at different seasons, displaying a wide range of origins, pathologies and requiring different times of hospitalisation, depending on their health condition.

Methods

For each animal, an extensive clinical examination will be carried out at arrival.

To test the **hypotheses 1 and 2**, we will:

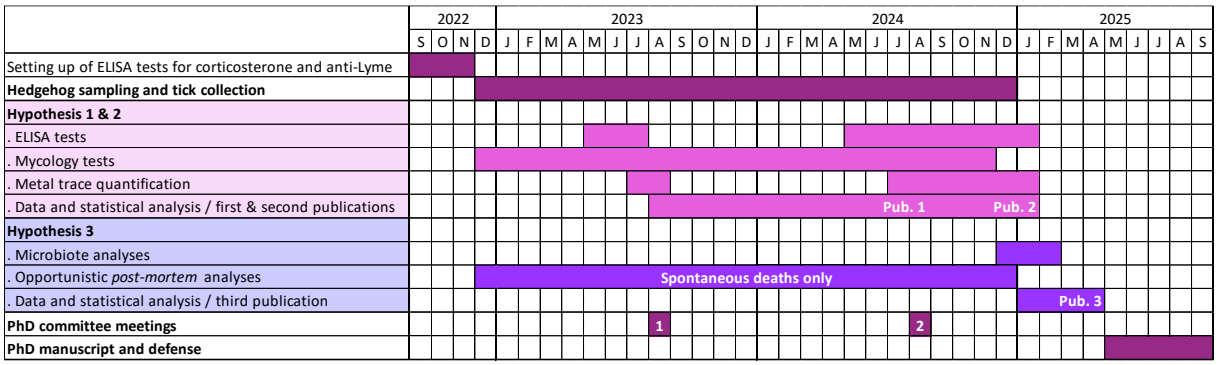
- Sample the hedgehog blood to estimate the seroprevalence of Lyme disease. We will also quantify baseline corticosterone level, a proxy of stress, in the blood using ELISA techniques, and in the faeces, a less invasive approach that is more suitable in a wildlife centre context.
- Evaluate the abundance and species of ticks found on hedgehog. Some of them (up to 3 by individual) will be collected to evaluate, using targeted molecular biology techniques, the presence of tick-born diseases.
- Sample the spines to evaluate the past individual trace metal exposure by quantifying their concentration in spines. Metals will be quantified through atomic absorption spectrometry after spine mineralization in nitric acid and hydrogen peroxide at high temperature. We will focus on 4 trace metals routinely measured in our laboratory: Cadmium Cd, Lead Pb (both toxic metals), Copper Cu and Zinc Zn (both oligo-elements) (Frantz et al. 2012).
- Sample the whole skin surface with special carpets (Le Barzic et al. 2020) to estimate the prevalence of *Trichophyton erinacei* carriage or clinical dermatophytosis.
- Estimate the degree of urbanisation of site of collection using data on land use method (Mode d'Occupation des Sols, MOS) provided online by the Institut d'Aménagement et d'Urbanisme Île-de-France (http://www.iau-idf.fr/), at different scales around the sites (100 to 1000 meters).

Collectively, these data will allow us to map the seroprevalence of pathogens and see the geographical distribution of the emerging pathogens within the region Ile-de-France (**hypothesis 1**). We will correlate the degree of urbanisation and trace metal exposure with the corticosterone levels (stress), the prevalence of ticks and ringworms and the seroprevalence of Lyme disease (**hypothesis 2**).

To test the **hypothesis 3**, we will investigate whether the baseline level of corticosterone, the seroconversion toward Lyme disease and the development of clinical dermatophytosis occur between the entrance and the release of hedgehogs. For this particular species the duration of hospitalisation can vary greatly depending on both cause and season of admission. In the winter, hedgehogs are indeed supposed to hibernate; the length of hospitalisation can be artificially prolonged as it is not possible to release them before the spring. This situation may deteriorate the quality of the hibernation and may induce additional stress. Our hypothesis is that these conditions (prolonged hospitalisation and altered hibernation) may have a negative impact on the hedgehog health condition. Another critical point that may have a negative impact is the hedgehog artificial nutrition. In the wildlife centres these insectivorous animals are fed with industrial croquettes which may affect their intestinal microbiota. We are planning to monitor the evolution of their intestinal microbiota and its putative impact on health by sampling some hedgehogs in a context of long-term hospitalisation.

We will finally carry out complete *post-mortem* analysis (necropsy, histopathology, immunohistochemistry, targeted molecular biology) for all the animals that will die during hospitalisation, to distinguish pathologies that are linked to the admission cause and those that are acquired during hospitalisation. In parallel, we will look for potential correlations between tissue damages and other pathogens in one hand and the degree of urbanisation and trace metal exposure on the other hand.

Provisional timetable



Interest and feasibility

This PhD project will benefit from the supervision of two researchers at the interface between ecology and veterinary sciences. The PhD will be hosted by the two different institutions for the different part of the project according to the expertise of each supervisor. For hedgehog follow up, sampling, and *post-mortem* analyses the PhD candidate will be hosted in the "Centre Hospitalier Universitaire Vétérinaire de la faune sauvage" (CHUV-FS) de l'Ecole nationale vétérinaire d'Alfort (EnvA). For plasma analyses (ELISA), trace metal quantification and statistical analyses, the PhD candidate will be hosted within the Institute of Ecology and Environmental Sciences of Paris (IEES-Paris) at Sorbonne University. The different methodologies used are mastered by the two different research teams and it guarantees the feasibility of data collection. This original project falls within two priority research domains of Sorbonne University: Ecological transition and One Health and will enable to open new areas of research based on interdisciplinary collaboration.

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