Staphylococcus aureus (SA) is a globally important opportunistic pathogen that colonises humans, livestock, wildlife, and companion animals[1]. While its ecology in clinical and agricultural settings is well characterised, surveillance in companion animals, especially cats, remains limited[2–4]. Most molecular studies have focused on dogs, despite reports of feline SA carriage rates up to 19%, often involving human-associated lineages and antibiotic resistance genes such as *mecA* and *blaZ*[3].

Existing studies primarily examine SA carriage in household pets[2,5,6]. In contrast, high-density, multispecies environments such as animal shelters remain largely unexamined. These settings may act as microbial reservoirs, facilitating persistent transmission, resistance dissemination, and local adaptation. To date, no studies have used whole-genome sequencing (WGS) or longitudinal molecular surveillance to investigate SA dynamics in shelter ecosystems, representing a major gap in One Health research[7].

This project builds directly on collaborative research with Dr Marta Matuszewska and Professor Lucy Weinert (University of Cambridge, Department of Veterinary Medicine), which involved genomic surveillance of SA in companion animals across Poland in 2021 (Kalinowski and Matuszewska, in prep). One component of this work focused on a multispecies shelter in eastern Poland, where cats were sampled during two visits (08/02/2021 and 15/04/2021). Among 85 samples, SA was isolated from 10 individuals (11.76%; 95% CI: 5.9–20.6%). Whole-genome sequencing revealed that 6 of these formed a tight phylogenetic cluster (≤89 SNPs), all belonging to clonal complex CC97. Bayesian MCMC analysis of a broader dataset placed the most recent common ancestor of this cluster ~15 years ago, supporting long-term local persistence. This is the first genomic evidence of SA persistence in a cat shelter in Poland and suggests that such environments may support conditions for microevolution and local maintenance of specific SA lineages.We now propose a focused, longitudinal study to build on these findings and directly test for SA persistence, strain continuity, and the emergence of novel genotypes within the same shelter. The pilot will assess whether genetically related strains, such as the previously identified CC97 cluster, continue to circulate among animals, the environment, and staff. Sampling will occur at three structured timepoints (months 0, 3, and 6), allowing detection of short- and mid-term transmission dynamics. At each visit, swabs will be collected from approximately 100 cats, 150 dogs, and 50 other resident animals, along with high-contact environmental surfaces (e.g. cages, feeding areas, litter trays). We will also include voluntary sampling of shelter staff or on-site veterinary personnel (self-sampling of shelter personnel will be conducted on a strictly voluntary and anonymous basis). All samples will be screened via PCR and allele-specific MLST (aMLST) to identify known sequence types[8]. All SA positive samples will undergo WGS and be analysed using well established and high-resolution phylogenetic and comparative genomic methods in collaboration with Dr Marta Matuszewska at the University of Cambridge[9]. This analysis will be conducted free of charge in collaboration with the University of Cambridge; therefore, the costs have not been included in the project budget.

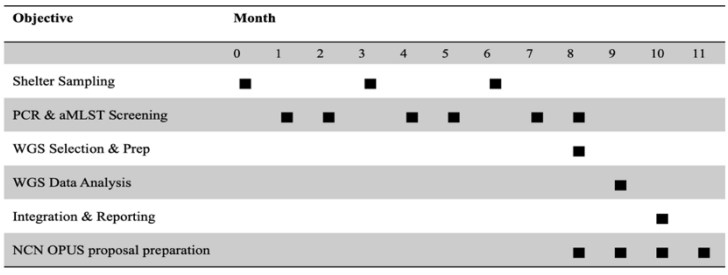

Figure 1. Miniatura Pilot Study Timeline. Visual overview of the 12-month pilot project investigating SA persistence in a multispecies shelter in eastern Poland. The timeline outlines key stages of the study, including three structured sampling visits (Months 0, 3, and 6), PCR and allele-specific MLST (aMLST) screening, whole-genome sequencing (WGS), data analysis, and final reporting and NCN OPUS proposal preparation.

Based on our previous prevalence estimate of 11.76%, we anticipate ~80 SA-positive isolates from the estimated 700 total samples across three visits. This sample size is sufficient to **(1)** detect recurrence of previously identified lineages (e.g. CC97), **(2)** assess strain turnover and emergence of new genotypes, **(3)** estimate substitution rates and identify shelter-specific adaptations (e.g. SNP accumulation, gene gain/loss), and **(4)** compare dynamics across animal species, environmental niches, and human carriers.

This study will generate the first high-resolution, time-series dataset on SA persistence in a shelter environment and provide a scalable model for structured pathogen surveillance. The resulting workflow and data will inform the design of a national One Health project across Polish voivodeships and support upcoming NCN OPUS and Horizon Europe applications. Beyond academic impact, this research will provide actionable insights into infection control, antimicrobial resistance, and zoonotic risk in shelter environments—supporting improved biosecurity and animal welfare policies.