

Methodology

Methodology – overview

Sixty Daubenton's bats, caught from the wild in England, will be held in an appropriate and humane laboratory context, and infected with an English isolate of EBLV-2 or used as non-infected controls. A number of virus challenge routes will be used to explore potential infection mechanisms and the development of lyssavirus pathology; all are potential routes of infection within wild populations of bats. The bats will be monitored continually to examine for detectable signs of infection (behaviour -scored on a predetermined scale, blood test for antibodies and saliva swabs for live virus). Bats will be held for a minimum of 120 days (30 days quarantine to establish baseline serology and at least 90 days during the challenge study) and would be humanely killed at the end of the study. If a bat shows signs of distress (especially those behaviours considered to be symptoms of a pathological lyssavirus infection), then it will be humanely killed to limit suffering.

In trying to ensure that we use bat / lyssavirus experimental models that most closely approximate the wild state in the UK, we feel that it is essential that wild English bats be used in conjunction with the EBLV-2 strain held at the VLA, one cultured and amplified from a wild English bat (██████████). Unfortunately, the experimental protocol requires that the bats be infected with EBLV-2, and that these bats must therefore be considered potentially infective. As a result, the bats must be held appropriately (i.e. in a biological isolation unit and at a level of security appropriate for rabies) for a considerable period (at least 90 days) and killed at the end of the experiment. The only research facility identified as capable and competent to perform this work is in Germany, necessitating the transport of the bats initially to the ██████████, for adaptation to captive life and then to the infectious challenge facility at ██████████ in ██████████. A small P3 challenge facility is available at the Veterinary Laboratories Agency but does not have the capacity to perform this study and continue statutory testing and research on rabies and rabies-related viruses.

Methodology – criteria for selecting roosts from which to remove bats

Bats will be taken from a number of roosts across all counties of England to minimise the disruption caused by removing individuals from communities. Roost selection will ensure that bats are not removed from roosts that are within 10km of each other, or if this is the case, appear to exist in distinct and separate communities separated by a geographical barrier. Alternative approaches to selecting roosts (i.e. the use of one large roost or of multiple roosts in the same community) are considered to pose risks to the viability of whole bat communities, which is considered unacceptable. Bats will not be taken from roosts known to be of ongoing interest to either local bat groups or other researchers,

Methodology – Capture of bats

Daubenton's bats will be caught in England using one or a combination of hand netting, cone trapping, harp trapping and mist netting. Ideally the bats retained for study should be adult females and so catching will be limited to a period during which

post-lactating bats can be taken from maternity roosts; typically the last week of July or the first few weeks of August (though this will depend upon a site assessment prior to catching). An experienced handler will check bats, and only those appearing healthy will be retained for study. The bats retained for study will be ringed with standard Mammal Society rings appropriate for Daubenton's bats (i.e. 2.9mm). The rings will be used to uniquely identify bats throughout the course of the experiment, and ensure that those bats caught and held but not used for the experimental work are returned to their capture sites. Individual bats will be selected for possession on the following basis:

- Adults will always be selected over juveniles for two reasons. They appear to have a more robust temperament and will adapt more readily to captive life. They are also likely to have a more consistent immunological experience given the relatively large span of ages for which a bat can be classed as juvenile.
- Females will always be selected over males, as they are less likely to fight through the course of the experiment. We have noted that bats can fight (and bite) during confinement, especially males during the breeding season. As part of the experiment protocol examines alternative infective pathways for rabies, biting (and therefore potential infection) is seen as a potential confounding factor and our design attempts to control for this. Some adult males can be used though they will not be deliberately mixed in experimental groups. Females considered to be pregnant or still lactating will not be selected. All unselected bats will be released immediately and returned to the roost.

More practically, with the very narrow window of opportunity between the adults females becoming definitely post-lactating, and the dissolving of the maternity roosts, there is likely to be more limited choice of bats than would be ideal, and despite the general principles we intend to apply I still expect the experimental cohort to represent a broad mix of ages and sex classes and not jeopardise the reproductive potential of any individual community. This will be especially true considering the requirement to sample across a number of roosts that will show distinct differences in community character. We are happy to conform to the requirement to limit the removal of no more than 25% of the adult females caught from any one roost, thereby ensuring that all sampled roosts can sustain population growth in future years and ensure subsequent population recovery.

Alternative seasonal timings for the collection of bats do not appear to exist. We feel that collection of bats from hibernacula, even if this were feasible, produces an unacceptably high level of disturbance on other hibernating Daubenton's and bats of other species, and considering the numbers required for the study, this would also be almost impossible. The collection of bats just after their emergence from hibernacula, when they are at their physiologically most vulnerable, we also consider unacceptable. In addition the collection of bats from after mid-March creates the complexity of some being pregnant which raised multiple ethical, practical and scientific issues, again which preclude this as a possible option. Thus the only window of opportunity once the young have been fledged is at the end of the breeding season whilst the adults are still available in the maternity roost.

Methodology – Housing bats

Although we intend to possess 60 bats for use in the experiment, there will be a need to capture and hold a higher number whilst the optimal cohort (in terms of age and sex composition) is assembled from a number of roosts across the country, We intend to limit the number that we would hold to a maximum of 120 Daubenton's bats. With those no longer considered suitable for the experimental work being released at their capture site as soon as possible. It is expected that bats would not be held for more than two weeks, and most are only likely to be held a few days, as most catching will be closely co-ordinated with their transfer to Germany. Bats will be housed in a wooden cage in a facility at [REDACTED], suitably adapted for bats, held at 20° C, and provided with separate bowls of mealworms and water *ad libitum*. The cage will be made escape proof and friendly to bats by including subdued lighting, cloth hangings etc and will be in a restricted area of the site ensuring quiet and security. However, as some bats can be slow to learn how to self-feed they will also be individually offered mealworms and water every two days to ensure that they do not loose condition,

Methodology – transit to Germany

We intend closely co-ordinate that catching, transport and housing activities and ensure that all licenses and permissions are in place to permit the rapid capture, and transport of the bats to Germany. The bats will be held together and transported, by plane, in a specially adapted piece of hand luggage to [REDACTED].

Note. This amended methodology was received by English Nature at 15:07 on 4/8/2006