REHABILITATION OF ENDANGERED BAT SPECIES. EVALUATING THE WILDLIFE RESERVOIRS RISK FACTORS, DISEASE MANAGEMENT AND POST-HANDLING SURVIVAL.

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Abstract

When handling endangered wildlife, one is faced with an ethical dilemma: is the potential risks associated with diseases greater than the survival of individuals? The relationship of host-pathogen is poorly understood in the case of most wildlife animals. Thus one must take into consideration not only the zoonotic risk when handling wildlife, but also the potential spill-over fenomen than can occur when the animals are released into the wild. Bats are well known for their capacity to host deadly pathogens without showing any clinical signs of illness. Thus when faced with a colony of protected bat species on a European, one must carefully evaluate the risks associated with the rehabilitation. This paper presents the medical dilemmas that appeared during the 3 months period while rehabilitating 4 different bat colonies consisting of a total of 142 individuals. At the end of the 3 months rehabilitation period, the survival rate reached 89%.

Key words: *bats, zoonoses, rehabilitation, rabies, leptospira*

INTRODUCTION

Each year, many veterinaries, biologists and volunteers carry out wildlife rescues and rehabilitation activities. Under the care of the specialists animals are treated and, if possible, released into the wild. Wildlife can harbor many zoonotic microorganisms, this risk should not be neglected giving the fact that rehabilitation workers constantly come into close contact with these animals. The potential transfer of these pathogens pose a great risk and should not be neglected. (Van Der Poel 2006, Sleeman 2006). Frequently more and more bats are submitted to local veterinary clinics. Despite their bad reputation and persecution, bats possess ecological benefits in controlling insect population. Several bat biodiversity awareness programs are conducted worldwide by local or multinational organizations (Bat conservation organizations UNEP-EUROBATS 2015).

Bats (ord. *Chiroptera*) hold the second most diverse order after *ord. Rodetia* and include over 20% of all mammal species. The tow suborders of bats are: *Microchiroptera* (*Yangochiroptera*) and *Megachiroptera (*Yinpterochiroptera). *Microchiroptera*, in response to low temperature will enter topor state which is a specific character similar to hibernation that regulates the basic metabolic rate, and body temperature. Bats are the only mammals capable of flying. Depending on their species bats can be frugivores, nectivores, sanguivores, carnivores, phytophagous or insectivores. (Simmons 2005). There are about 1240 species distributed worldwide, with 45 different bat species living in Europe. All european bats with one exception (*Egyptian fruit bat),* eat beetles, flies, moths and other insects.

The ecological and economical importance of bats is given by their critical role in controlling the insect populations. A single bat can eat up to 1,200 insects every hour. Although their environmental role is undisputed, bats can also host a wide range of viruses, bacteria, rikettsia and mycoses (Wong 2007, Smith 2013, Mackenzie 2013). The virus replication process is dependent on the body temperature. The relationship between pathogens and topor or hibernation in terms of maintenance of viral infections is still unclear. However, there have been reports of persistently infected bats with *Lyssaviruses* or *Flaviviruses* (Sulkin 1974). Antibody studies also sustain this hypothesis (Kuno 2001)

More than 60% of the 335 disease events recorded in the past 6 decades were zoonoses, the origin of 72% of these events was related to wildlife populations (Gummow 2010). When comparing the total amount of zoonotic agents present in bats (ord *Chiroptera*) and in rodents (ord. *Rodentia*), bats

received increasing attention for their unique role as potential reservoirs for zoonotic agents (Luis 2013). At a macro scale, the human expansion along with close contact with wildlife populations are in fact the driving factors of the emergence of zoonotic diseases (Cunningham 2005). At a micro scale, molecular changes may contribute to spill-over events if and when mutations or recombination occur (Morens 2004). The pathogens can have a key role in the structure and size of their host population. Many pathogens are indeed capable of infecting multiple host species, but at a different extent.

Pathogens can have a key role in the structure and population size of their animal hosts. Up to the host susceptibility, are possible different outcomes: a pathogen could be even cleared, while someone could establish a persistent equilibrium with their host and be shed. In some cases, pathogens can lead to fall the host population density below a threshold necessary to establish a continuous spread (Anderson 1991). While rehabilitating bat species, those possible outcomes can have a major impact over the chosen release area. If the individuals have a subclinical infection then they might disseminate pathogens into a disease-free area.

Transmission of zoonotic infectious agents from bats to humans can occur in various ways: by direct contact, by bites, by scratches, by inhalation of infectious particles (these could be infectious aerosols resulting from saliva or guano) etc. These data lead to the need to establish the implications of microbial agents in order to obtain data to substantiate adequate preventive measures to limit the risk of infection. However, except for rabies, most viruses found in bats occur only in specific regions of the globe and seem to be related to these. (Sonya R. 1995).

The limited knowledge and perspective about pathogen diversity and susceptibility in wildlife populations tend to cloud the so-called One Health vision. Most models fail to acknowledge the fact that some spillover events can have their origins in humans that shred pathogens into wildlife populations (Lloyd-Smith 2009). In practice, the same factors that enable zoonotic transmission from wildlife to humans can lead to a wildlife outbreak of “human” pathogens. Furthermore, the host-pathogen co-evolutionary history adaptation might cause in most species inapparent infections in terms of overt disease but in the same time could cause frank disease in certain species. This may enhance the chance of a human to wildlife spillover event.

Urbanization is increasing at a global scale, and obvious patterns of biodiversity loss are observed. However, multiple taxonomic groups managed to adapt to simplified habitat structures and resource availability. Those urban-adapted species are influenced by the contact patterns and by the spatial distribution of resources. These are the key factors that can determine the outcome of some infectious diseases through the so-called “dilution effect”. Chronic stress along with heavy metal and pesticide pollutants can lower resistance to infection (Bradley 2006).

Our objective was to rehabilitate and release the bats while minimizing the unnecessary stress of handling. The goal was to do this in a manner that will not cause or spread disease. Through emerging infectious diseases, therefore, implementing a common agenda should be a priority for the medical, veterinary and wildlife conservation professions, under the same One Health principles.

MATERIALS AND METHODS

Health status of the animals

In winter 2015 *Centrul de Reabilitare a Animalelor Luana* recived a total of 142 bats. These included *Nyctalus sp* and *Myotis sp*. All bats were rescued from a construction site located in a highly populated area in the civic center (N:44.4536, W:26.0832). On admission, all bats were given a general physical examination. All the bats were identified then individually marked using approved 4.2mm magnesium-aluminum flanged rings. The following data were recorded for each individual: ring id, species, sex, body weight prior to feeding, body weight after feeding, when and where the bat was found, clinical presentation, medications, final outcome (died or survived), findings of gross necropsy, findings of microbiology, staff bitten while handling bats, observations. After evaluating the general condition of the bats, it has been justly observed that most individuals had signs of emaciation and dehydratation. In several individuals, ticks were observed and manually removed and then treated with

topical fipronil (Frontline). Severe traumatic injuries were noticed in 5 individuals: 1 of them had a distal humerus open fracture and the other 4 had close-fractured of the radius. The bats with minor injuries were hold in quarantine, standard precaution protocol, and received one dose of 1.0ml warmed LRS subcutaneous, followed by 2.0ml/day Duphalyte (Fort Dodge) per os. The bats with major injuries were examined using plain Radiography, standard whole-body antero-posterior position, in order to evaluate the fracture topography. The bats were premedicated with 5mg/gk Butorphanol IM then induced and maintained with 5% Isofluran. After re-aligning the bone fragments to their normal anatomical position, the wing was immobilized using a Cyanoacrylate adhesive. Analgesia was maintained for 5 days. Complete callus formation was observed for all close-fractured lesions after 25days.

Quarantine parameters and Sample collection

To avoid heat exhaustion, all the bats were kept in a controlled environment. Using a Innova CO48 incubator with constant levels of temperature of 22ºC, constant humidity range 50-55%, constant levels of CO_2 with a 25% fresh air exchange every 10 minutes and restricted light input to reduce stress. While in the controlled environment of the incubator, bats entered a torpor state. It was noted that the average temperature of the bats hold in the incubator was on average +4ºC above the temperature of the chamber. During this torpor state, the bats were mostly dormant. Body weight was taken into consideration to design a feeding pattern. For this purpose, body weight was recorded for each individual, prior to feeding and after feeding. Despite good appetite and fluid intake, most of the bats continued to show signs of dehydratation and emaciation. After 3 days in quarantine, one of the bats dies. Among available diagnostic tools, anatomic pathology is highly effective in recognizing or excluding infectious diseases based on morphological changes in affected systems or organs. The necropsy revealed lesions suggesting kidney failure, so we decided to investigate further and test for *Leptospira sp*.

The bats from *Centrul de Reabilitare a Animalelor Luana* had to be tested for Rabies, standard protocol, and also had to be evaluated in terms of general condition. Samples were collected using nonlethal-noninvasive methods. Engloser sterile swab stick moisted with 10 µl RNAlater stabilization solution was wiped inside the mouth of each bat and was processed within 24h of collection. For urine sampling, we used a 20µl single channel micro pipette and 0.5ml Eppendorf Safe-lock tubes.

Assessment of pathogens on quarantined individuals

Detection of rabid bats is a matter of public health concern due to the risk associated with exposure of the staff while handling the animals. While in quarantine, oral swabs were collected in order to determine the presence or absence of *Lyssavirus* in saliva using molecular biology techniques, standard protocol. All the dead individuals were tested for rabies at the Institute of Diagnosis and Animal Health. All the samples were tested using fluorescent antibody technique and mouse inoculation tests, all results were negative.

Rabies virus neutralization test was also performed in order to detect post exposure rabies antibodies. To avoid unnecessary stress, the Virus Neutralization test was performed on 10 specimens, each weighing more than 30g. A cardiac puncture (Kunz 1988) was performed using a 3/10 mL/cc insulin syringe with half-unit markings and a 0.4 by 12mm needle. It was extracted a total of 0.150mL of blood to yield 0.05mL of plasma per individual. Each individual was monitored for 15 minutes after the procedure. No antibodies were detected in any of the tested specimens. Because of their protected status, cardiac puncture should be performed on maximum10% of a bat colony. In endangered species we recommend other means of testing disease like molecular biology, if suitable.

All medical procedures, sampling, feeding, handling and subsequent work reported here was carried out by professionals previously immunized against rabies. All laboratory procedures involving potentially infectious material were conducted in a biological safety cabinet (Aquaria Vertical laminar flow Biohazard cabinet Class II-A/B3 Type).

After one week in quarantine, urine samples were taken and analyzed using IDEXX analyzer to evaluate potential signals of kidney damage. Most of the parameters were in the normal range of the

analyzer. Due to the fact that there were no specific range and procedural method for bat parameters, the acceptable values of calibration were set to the ones of lab mice because of their similar weight. The renal parameters were not different between the 4 different bat colonies that were under quarantine. It has been noticed a high glucose level in all the individuals. This might be an indicator of handling stress, typically expressed by small animals and most wildlife.

At the gross necropsy one of the individuals expressed signs of kidney failure. The etiology of this lesion was later confirmed using the silver staining technique that revealed the presence of *Leptospira* in the kidney tissue. *Leptospira* was also identified in the urine of some of the remaining individuals, using dark-field microscopy. Since dark field microscopy test and silver staining might give false negative results, we tested the urine of all survival individuals *via* molecular biology techniques. Using classic PCR primers and method as described with the following sequences: A, 5'-GGCGGCGCGTCTITAAACATG-3'; B, 5'-TTCCCCCCATTGAGCAAGATT-3'; C, 5'-CAAGTCAAGCGGAGTAGCA A-3'; and D, 5'-CTTAACCTGCTGCCTCCCGTA-3'. Using a DNA thermal cycler (PCR sprint) the following protocol was used: denaturation 94°C for 3minutes, followed by 35 successive cycles of denaturation 94°C-1minute, annealing 63°C- 1.5minutes and extension 72°C- 2 minutes. At the end of the 35 successive cycles, in order to complete the primer extension an additional 72°C -10 minutes was added. All the surviving individuals were treated with 0.005 mL/g doxiciclin for seven days. We repeated the tests at 21 days after treatment and the results were negative.

Release site – challenges

Bats are known to be faithful to their roosts, and are likely to return in the same area. Also they apparently can travel up to 10km from their roosts to find food. While looking for a suitable release area, the Botanical Garden had all the key features in terms of biodiversity (N: 44.4351; W: 26.0611). The area offered ecologically valuable biotopes with natural and semi-natural environment. Also the strategic location in a less built-up area and a relatively long distance from the area where the bats were rescued from made it suitable as a release area. The location comprises the Botanical Garden about 18ha, and the surrounding areas with a dense vegetation cover, 3km^2 in total. A special bat house was build that mimics the craves between of the building where they were found and mounted in one of the trees.

In this case, finding a suitable relocation site for the bats and minimizing the risks of pathogen dissemination in the environment is a high priority. Prior to release, all the bats were vaccinated subcutaneously with 0.5ml ml of Biocan RL vaccine (inactivated *rabies virus*, inactivated *L icterohaemorragiae,* inactivated *L canicola,* inactivated *L grippotyphosa*)

RESULTS AND DISCUSSIONS

At the end of the 3 months rehabilitation period, the survival rate was 89%. The low incidence of significant clinical findings associated with traumatic lesions 2% and feeding refusal 3,4% suggests that most individuals were healthy at the time of rescue. The results show also that bats are highly sensible to temperature variations during their hibernation. In response to increased temperatures, bats will leave their roosting habitats and will die from depletion and lack of food. These results indicate the need of intervention and rehabilitation when such episodes occur. The conservation value of this rehabilitation program is likely to be highly successful.

Based on serologic results, the bats that were roosting in the city center did not come into contact with rabies virus (lack of antibodies). This hypothesis is sustained by the absence of the antigen in saliva using classic PCR and by the negative results of the fluorescent antibody test.

Our findings confirm the presence of *Leptospira* in one of the four clusters that were sheltered to the Center. Since *Leptospira* is known to be transmitted by the rodent urine, that contaminates water, sharing biotopes with the rodent populations increases the probability for the spread of of this pathogen to city resident bats. The positive results for *Leptospira* sp, in some specimens from the same cluster suggest that bats, occasionally, may become infected with this pathogen, most likely by the

contaminated water. The key issue in this case is identifying reservoir species and taking proper actions to minimize pathogen dissemination.

Based on our findings, bats with traumatic lesions at a humeral or radial level have a less chance of survival if the fracture is opened because of the risk of septicemia. If the fracture is complete but closed, we recommend re-aligning the bone fragments to their normal anatomical position and gluing the wing with a non-toxic adhesive (*Cyanoacrylate*). The fix bandages or the synthetic adhesive material should be avoided since pain along with foreign objects are stimuli for self-mutilation.

The dilemma regarding the energy requirements in the torpor state had a simple solution: we treated the colony as one individual and weight them all. When the total weight dropped by 150g (1g per individual) then the bats could be woken and fed. This reduces the discomfort of handling and the stress associated with repeated wakening.

After releasing the bats, we kept on monitoring them for 1 month. Approximately 50% of the bats remained in the new roost, most of them were females. This could be an indicator that these bats did find sufficient resources in the relocation area. Some males were reported in the original building or in the proximity of the original place from where they were first rescued. Thus, it appears that they managed to find their way back going through relatively long distance from the Botanical Garden to the city center. The complex mechanisms that triggered some bats to fly back to the original roosting place while others adapted into their new environment is still unknown.

The risk of a spillover phenomenon must be treated seriously and for this, every precaution must be taken. We recommend another set of examination prior to release and vaccination of all wildlife animals that came into a rehabilitation center.

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