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Original Investigation

# Effects of sewage-water contamination on the immune response of a desert bat

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ABSTRACT

Environmental pollutants may negatively affect the immune system of animals. Yet, this phenomenon has not been studied thoroughly in terrestrial animals that use polluted water for drinking and/or foraging. We experimentally tested the hypothesis that exposure to sewage water would affect the activation of the immune response in the bat *Pipistrellus kuhlii* that drinks from bodies of open water. We selected two water sources where bats forage in the Negev desert, Israel: natural springs and a sewage-polluted man-made reservoir. We captured 13 non-reproductive female bats in the vicinity of the natural springs and offered seven of them water from the sewage-polluted source for 30 days (treatment) and the remaining six bats were offered water from the natural spring (control). Consumption of contaminated water did not alter the bactericidal ability of blood plasma or the proportions of monocytes circulating in the blood. However, our data provided evidence that the 30-day treatment can cause a decrease in the relative levels of neutrophils and an increase in the levels of lymphocytes. Our study provides a first account for the effect of sewage pollution on bat immune response which may be important in desert environments, where water sources are scarce. We suggest hypotheses for future, more focused studies.


## Introduction

Anthropogenic habitat degradation or pollution can negatively affect animal health (Anderson and Maldonado-Ocampo, 2011). In particular, environmental pollutants may compromise the immune system of animals, as has been shown in a variety of taxa (e.g. Rohr and McCoy, 2010). Aquatic environments are highly sensitive to human-induced contamination (Schwarzenbach et al., 2006). Correspondingly, the effects of water contamination on vertebrate immune systems have been studied mainly in aquatic organisms (Christin et al., 2003; Milla et al., 2011). Nonetheless, the immune system of terrestrial animals that use water for drinking or bodies of open water for foraging may also be affected by pollutants (Conrad et al., 2005; Kozul et al., 2009). The risk to animal health may be exacerbated in desert environments where water is scarce and animals may be obliged to use vital but contaminated water sources. Although water pollution may have detrimental effects on desert mammals (O'Shea et al., 2001), direct evidence for the effect of drinking water quality on the immune response of mammals in desert environments is lacking.

The vertebrate immune system is composed of innate and adaptive lines of defense. The innate immune system is non-specific and includes various phagocytic cells such as neutrophils and monocytes that engulf and kill foreign cells, and are recruited in large numbers upon infection (Weiss and Wardrop, 2010). The complement component of the innate immune system involves proteins that lyse foreign cells, trigger the recruitment of inflammatory cells as well as alert other aspects of the immune system, including cells involved in memory responses (Song et al., 2000). The adaptive immune system is specific in nature and its responses are carried out by T and B lymphocytes, which are usually activated by signals received from the innate immune system (Weiss and Wardrop, 2010).

Monitoring the composition of white blood cells (WBC) is a useful tool for ecologists who aim to detect effects of external factors on vertebrate immune response (Davis et al., 2008). Sewage effluents contain a mixture of abiotic and biotic pollutants, which may be manifested by changes in the composition of WBC (Maceda-Veiga et al., 2010). An effect of sewage effluents on immune response may thus be detected by analyzing leukocyte profiles expressed as differential WBC counts (i.e. proportion of different WBC types) prior to and after treatment with polluted water. Indeed, differential

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WBC counts were previously used to assess vertebrate immune response to various factors including sewage pollution (Suorsa et al., 2004; Cottontail et al., 2009; Maceda-Veiga et al., 2010). This approach can be supplemented by using additional indices of immune function such as measuring bacterial killing ability (BKA) of complement proteins in blood plasma – a functional measurement of innate immune response (Tieleman et al., 2005).

In Israel, bats are a sensitive group with high conservation priorities. In addition, insectivorous bats can be excellent bioindicators of habitat quality, including water quality (Jones et al., 2009). Some bats are more tolerant than others to sewage-contaminated foraging environments as seen from bat activity (Park and Cristinacce, 2006; Kalcounis-Rueppell et al., 2007; Abbott et al., 2009) but no study that we know of has examined the effect of sewage water on bat immune function. Here, we tested the effect of sewage water on the immune response of the insectivorous bat *Pipistrellus kuhlii* (Kuhl, 1819). *Pipistrellus kuhlii* uses natural and man-made water sources (e.g., artificial ponds) as foraging and drinking sites (Korine and Pinshow, 2004).

Our goal was to estimate the effect of sewage-pollution on bat immune response in order to provide a basis for future studies. We hypothesized that a prolonged exposure to sewage water would alter the immune response of bats. We tested this hypothesis by offering sewage-polluted water to bats captured in a natural, unpolluted site and measuring their immune response to the treatment.

## Material and methods

### *Study sites and water analysis*

The study was done in the Negev Desert Highlands, Israel, where water sources are scarce and spatially separated. We selected two sites which were permanent water sources rich with insects. The first, clean, site was located 2–3 km from the national park Ein Avdat (30°50′N, 34°53′E), where bats forage over a complex of natural ponds (Razgour et al., 2010). The second, polluted, site was the Yeruham Reservoir (30°59′N, 34°53′E), an artificial reservoir used by bats for drinking and foraging (Korine and Pinshow, 2004). During the course of this study Yeruham reservoir was receiving untreated sewage water.

To estimate the quality of the water at the two study sites, we collected water at one representative pond in Ein Avdat (20 × 5 m) and around the shore of Yeruham Reservoir, once a month from June to August. During each sampling period, we collected the water at 3–4 locations around the Ein Avdat pool and at 7–10 locations around the shore of the reservoir (distanced ca. 100 m from each other), where bats were observed foraging and drinking. To verify a proper selection of the two sites we estimated the general differences in water quality between them. Specifically, we measured four indices of water quality: chemical oxygen demand (COD), biological oxygen demand (BOD), fecal coliforms (FC) and total suspended solids (TSS). These are commonly used indices for biotic water analysis, with well-established and standard methodology (Nollet, 2007). High values of these indices indicate sewage pollution and generally low water quality. In particular, fecal coliforms are a good proxy for the presence of pathogenic bacteria, at least for humans (Nollet, 2007). All water analyses were done at the Zuckerberg Institute for Water Research, Ben-Gurion University of the Negev.

### *Experimental design*

We tested the immune response of bats captured at the clean site but exposed to water from the polluted site. Using mist nets, we captured 13 non-reproductive female *P. kuhlii* in the clean site during July 2010. Despite extensive efforts, very low capture success rates impeded us from collecting more bats and from collecting bats at the polluted site. During acclimation to captivity (5–14 days), bats were trained to eat mealworms (*Tenebrio molitor* larvae) independently and were offered water ad libitum from the clean site. After the end of the acclimation period (day 0 for the experiment), we randomly assigned each of the 13 bats to one of two groups: control (bats offered water from clean site; $n = 6$) and treatment (bats offered water from polluted site; $n = 7$). During acclimation, the bats were housed as a single group. For the duration of the experiment, they were housed in two separate groups, control and treatment, in the same outdoor aviaries.

To examine the association between water quality and immune function we measured (1) leukocyte profiles using differential WBC count and (2) BKA of plasma at day 0 and after 30 days of exposure to water (day 30) for all bats. We stored the water from each site at 4 °C because this is the temperature at which bacterial samples are usually kept for frequent use. We administered water at room temperature to the bats and replaced it every other day with fresh water collected from the two sites.

During the 30 days of the experiment (between day 0 and day 30), bats were fed mealworms ad libitum and their body mass was measured daily with a digital balance (PPS200, Pesola, Switzerland, ±0.01 g). We calculated a body condition index as the residuals of an ordinary least squares linear regression of body mass against forearm length (Reynolds and Korine, 2009). To monitor individual differences in water consumption, we gave the bats water (separately for each individual) on a daily basis and recorded the volume of water consumed by each bat with an accuracy of ±5 μl.

On days 0 and 30 we collected 10–20 μl of blood from each bat in a heparinized capillary (75 μl capacity, Marienfeld, Lauda-Königshofen, Germany) by puncturing the cephalic vein with a 27 G needle (Voigt and Cruz-Neto, 2009). We immediately used whole blood to prepare blood smears, centrifuged (Hettich Mikro-22R, GMI Inc., USA) the remainder for 10 min at 3000 rpm to separate plasma from formed elements, and stored plasma at −80 °C until BKA assays were performed. Samples of control and treatment groups were frozen for the same amount of time (3–7 days).

Bat captures and experimental procedures were reviewed and approved by appropriate committees and were conducted under license # 34615 given to CK by the Israel Nature and Park Authority (for animal captures) and license #BGU-R-02-2009 given to SP (for animal care protocol). We released all the animals after the experiment at the site of capture.

### *Immunological assays*

We prepared blood smears using the wedge technique (Brown, 1993) and staining with an eosin-thiazine stain (Dip-Quick Stain Set, Product #J322, Jorgensen Laboratories, USA). We analyzed the WBC profile of the bats using the cross-sectional method of differential counting (Brown, 1993). For each bat, we examined 2–4 smears under a ×1000 magnification, until a total of 200 WBC per smear were counted. Counts were averaged across smears. We differentiated between neutrophils, lymphocytes, monocytes, eosinophils and basophils based on cell morphology (O'Connor, 1984; Brown, 1993).

Our BKA assay quantified the killing ability of bat plasma and followed Moore et al. (2011). Briefly, we made a stock of diluted *Escherichia coli* (ATCC #8739, E$^{power}$ Microorganisms #0483E7, MicroBiologics, St. Cloud, MN, USA) that would result in the formation of 200–300 colony forming units when 50 μl aliquots were plated on agar. The killing ability of the blood plasma was compared to that of a control in the following way. Control vials were prepared by mixing 60 μl of the *E. coli* stock in 840 μl media [5 ml $CO_2$-independent media (product # 10010023, Invitrogen, CA, USA)