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NITROGEN METABOLIC WASTES DO NOT INFLUENCE DRINKING WATER PREFERENCE IN FERAL PIGEONS

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Pathogens of both avian and mammalian fecal origin can infect birds via drinking water. Since birds often use ultraviolet clues for many decisions such as food detection, they may also be able to distinguish between faeces- or urine-polluted water and clean water by using UV vision. Here we test the hypothesis that birds may assess potential pollutions in drinking water bodies using UV absorptions/reflections. Feral pigeons were offered with (1) clean tapwater, (2) uric acid polluted water (UV-absorbant) to mimic wastes of avian origin and (3) urea solution (UV- reflectant) to mimic wastes of mammal origin. Contrary to our expectations, pigeons exhibited no detectable preferences in double-choice experiments.

Key words: pigeon, nitrogen metabolism, water preference, UV-vision, pathogen

INTRODUCTION

Pathogens often influence their hosts' preferences when selecting a suitable habitat, nest site or sexual partner (APANIUS & SHAD 1994, HUTCHINGS & HARRIS 1997). For instance, mate preference might be influenced by the danger of infections directly transmitted by sexual partners during copulations (MØLLER 1990). Similarly, food preferences are known to be affected by potential food-borne infections in a number of bird and mammal species. Both oystercatchers *(Haematopus ostralegus)* and sheep *(Ovis aries)* face a choice between feeding on pathogen-free food with little nutritive value or feeding on nutrient-rich but infected food items (HUTCHINGS *et al.* 1999, NORRIS 1999).

Drinking water preferences might also be important components of hygienic adaptations of birds. Birders' guides often emphasize that the drinking water supply for garden birds has to be re-freshed at least daily since birds often defecate into it (see e.g. MIZEJEWSKI 2004). Arguably, fecally contaminated water bodies pose a real threat for birds living in dry habitats and using only a few small and ephemeral water bodies as sources of drinking water shared by many individuals. Avian excrements in drinking water evidently pose a health risk for birds as they can serve as a transmission route to a variety of avian pathogens such as *Salmonella* spp., *Mycobacterium avium*, influenza viruses (STALLKNECHT *et al.* 1990) or Newcastle disease (AWAN *et al.* 1994). Mammalian excrements were also found to be potential hazards to birds e.g. by carrying *Salmonella typhimurium* or *Toxoplasma gondii* propagules (MACKINTOSH *et al.* 2004, REFSUM *et al.* 2003, DUBEY *et al.* 2004). Therefore, birds should benefit from detecting and avoiding excrement-polluted drinking water.

However, the view that drinking water actually carries a variety of avian pathogens in natural habitats is rarely supported by empirical data in the literature. Therefore, to show that feral pigeons (*Columba livia* L.) used in our experiments also face the risk of water-borne infections we aimed firstly to collect water samples from puddles of urban squares and analyze their microbiological quality.

Birds and their relatives use UV clues for a variety of purposes (BENNETT & CUTHILL 1993, CUTHILL *et al.* 2000, CUTHILL 2006) including orientation (KREITHEN & EISNER 1978), species recognition (FLEISHMAN *et al.* 1993, BLEIWEISS 2004, DALTON 2004), intraspecific communication (BURKHARDT 1989, BENNETT *et al.* 1996, HUNT *et al.* 2001, PEARN *et al.* 2003, BLEIWEISS 2004) and even for prey detection (BURKHARDT 1982, LEE *et al.* 1990, VIITALA *et al.* 1995, HONKAVAARA *et al.* 2002). The principal waste-product of avian nitrogen-metabolism is uric acid. It remains mostly undissolved in water as a white suspension that absorbs UV light. The pigeons' spectral sensitivity determined behaviorally falls between 320 and 640 nm (EMMERTON & REMY 1983). Though the absorption maximum of uric acid is at 286 nm (RINGVOLD *et al.* 2000), its relative absorption is still 0.05 at 320 nm (G. O. personal measurements), the extreme end of pigeons' UV-A sensitivity. The nitrogen waste-product in mammals is urea (carbamid). Urea easily dissolves in water and reflect the UV-light (VIITALA *et al.* 1995). Though mammalian urine itself carries a few if any pathogens, however, we presume that urine pollution is likely to indicate the presence of fecal pollution too. Thus it seems conceivable that birds can assess drinking water quality by using both visible and UV light clues produced by these wastes.

To our best knowledge, drinking water preferences and their hygienic consequences have not yet been studied in birds. We presume that birds can either detect avian N-metabolism wastes by using visible light or detect both avian and mammalian wastes using UV light. Here, we test avian drinking water preferences using the two extreme ends of the UV darkness-lightness continuum; a uric acid suspension (UV dark, to mimic avian-borne pathogens) and an urea solution (UV fluorescent, to mimic mammalian-borne pathogens) against clean water control.

MATERIALS AND METHODS

To identify potential water-borne pathogens in urban puddles, 20 water samples were collected in Budapest from different squares inhabited by pigeons. We observed pigeons visiting, drinking and defecating at these puddles several times. From each place we collected samples for parasitological (0.3 l sample + 0.2 l ethanol) and for bacteriological (0.5 l sample in sterilized bottle) analyses.

We investigated drinking water choice in captive feral pigeons in July and August, 2005 at the Budapest Zoo & Botanical Garden, Hungary. Pigeons often share small and fecally contaminated drinking water sources (G. O. and L. R., personal observations) and they have an advanced UV vision (EMMERTON & REMY 1983). Fifty feral pigeons were captured in Budapest and kept in outdoor aviaries of 5 × 4 × 3 m (L × W × H) in two flocks. All birds were individually marked. Pigeons were fed ad libidum with dried bread, barley, maize and sunflower seeds. We observed single individuals' drinking water preference between (i) uric acid suspension and tap water (experiment 1), (ii) urea solution and tap water (experiment 2) and (iii) uric acid suspension and urea solution (experiment 3). We tested whether pigeons discriminate uric acid suspension against urea solution because these molecules represent the extreme ends of the UV darkness-lightness continuum.

Each experimental bird was chosen randomly and transferred into an outdoor test aviary of 5 × 1 × 3 m (L × W × H). Experiments were done only when at least a part of the sky was free of clouds. Before the start of each experiment, focal pigeons were deprived of water for about 24 h. At the start of the experiments we offered drinks in two identical, brown plastic plates (flowerpot underplates) to each bird. After each day, the plates were cleaned carefully with pure water, so they remained identical in the entire experiment. The two plates were placed symmetrically and at equal distances from the focal bird's perch. Left and right hand positions of test drinks were randomly selected for each bird. Solutions and suspensions were freshly made every morning. Each bird was observed for a maximum of 10 minutes and only the first drink was taken into consideration. After the first drinking event we stopped observations. Focal pigeons were then transferred into a 3rd aviary. Birds in one aviary could not see birds in the other aviaries. In experiment 1 and 2 we used 25 birds of the first flock. Each bird was used two times in both experiments. In experiment 3 we used 20 birds of the second flock, and again, each bird was used 2 times. At least one day elapsed between two trials for each bird in all the experiments.

We used uric acid suspension (0.5 g/l, Aldrich, 99+ %) and urea solution (10 g/l, Reanal, a.r.) with tap water as solvent to mimic metabolic wastes and excluded the effect of any other potential pollutants. These drinks were offered against tap water control. Concentration levels were based on arbitrary decisions and aimed to represent very strong levels of fecal or urine pollutions by birds or mammals, respectively. Considering that birds' excrement contains about 9% uric acid (COLUMBIA ENCYCLOPEDIA 2006), the suspension used in the experiments matches about 6 g faeces (dry material) mixed in 1 liter of water. Higher concentrations of uric acid might have provoked stronger effect; however, it would also look like a milky suspension visible to the naked human eye. On the other hand, urea dissolves in water easily, and the concentration used in our experiments is about 13 times higher than that of human urine (0.6–0.9 g/l) (University of Pennsylvania Health System 2004).

For the statistical analyses of the experiments we used binomial tests (test proportion: 0.50) to detect potential deviations from expected pattern of random (50–50%) choice. Fisher's exact test was used to detect the independence between water and side preferences. Data were analyzed by SPSS 11.0 for PC. All tests are 2-tailed. Asymptotic P-values are given.

RESULTS

In the course of the bacteriological and parasitological analyses, *Eimeria* spp. sporulated oocysts, *Capillaria* spp. and *Trematode* eggs, *Salmonella* spp., *Yersinia* spp., *Clostridium* spp. and *Escherichia coli* bacteria were identified in some of the samples.

In experiment 1, pigeons chose clean tap water in 24 cases and uric acid suspension in 26 cases. Similarly, in experiment 2, pigeons chose clean tap water in 26 cases and they drank urea solution in 24 cases. None of these results differ from a random outcome (binomial test, P = 0.888 for both cases).

In experiment 3, pigeons drank uric acid suspension in 19 cases and drank urea solution in 21 cases. The proportion of these decisions fits closely to the 50–50% proportions expected by chance (binomial test, P = 0.874).

On the other hand, however, a significant side preference was observed in the first two experiments (experiment 1: 12 versus 38, and experiment 2: 11 versus 39 on the right versus left side; binomial test, P = 0.000 for both cases). This side preference was independent of the birds' decisions about which drink to choose in every experiment (Fisher's exact test for the three experiments: $P_{exp1} = 0.190$, $P_{exp2} = 0.738$, $P_{exp3} = 1.000$).

DISCUSSION

Among the four chemical elements (CHON) that build up the majority of all living materials, only N is likely to come together with metabolic wastes potentially detectable in drinking water. Our results indicate that urban puddles – a major source of drinking water for feral pigeons – are rich in water-borne pathogens potentially harmful for pigeons. The presence of *Salmonella* spp., causing paratyphus avium, *Yersinia* spp. causing yersiniosis, and *Clostridium* spp. causing botulism and tetanus in birds is particularly appealing. Furthermore, *Eimeria spp.*, *Capillaria* spp. and unidentified Trematode eggs also pose a risk of water-borne infections in urban habitats. Therefore, we hypothesised that birds use N-wastes as clues to identify fecal pollutions is drinking water.

Contrary to our expectations, however, our results do not support the hypothesis that pigeons base their drinking choices on the presence of N-metabolic wastes in water bodies. Even using extremely large (particularly in case of urea) doses of these wastes provoked no behavioural response from feral pigeons. The solutes may have been more detectable in light-coloured containers (e.g. aluminium, which has flat reflectance across the pigeon-visible spectrum). Using brown water bowls is a perfectly valid method as many natural backgrounds are dark. According to the literature cited above, both urea and uric acid has a characteristic UV colouration visible for pigeons, and uric acid was even visible in the suspension for the naked human eye. This apparent lack of response was not resulted by the side preference observed in experiment 1 and 2, as the side preference was not absolute and pigeons chose any type of drinks with the same chance on both sides. At least two factors may contribute to this unexpected result.

First, urban habitats may be richer in drinking sites than we presumed. Thus feral pigeons may not necessarily be selected to develop fine-tuned adaptations to avoid polluted sources of drinking water. Second, birds may use clues other than the wastes of N-metabolism to detect fecal pollutions, e.g. the direct observation of defecating individuals or other components of the faeces itself.

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