Science of the Total Environment 508 (2015) 297–306

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Sources of variation in innate immunity in great tit nestlings living along a metal pollution gradient: An individual-based approach

Anke Vermeulen [a,*], Wendt Müller [a], Kevin D. Matson [b,d], B. Irene Tieleman [b], Lieven Bervoets [c], Marcel Eens [a]

[a] Department of Biology — Ethology, University of Antwerp, Universiteitsplein 1, 2610 Wilrijk, Belgium
[b] Animal Ecology Group, Centre for Ecological and Evolutionary Studies, University of Groningen, P.O. Box 11103, 9700 CC Groningen, The Netherlands
[c] Department of Biology — SPHERE, University of Antwerp, Groenenborgerlaan 171, 2020 Antwerpen, Belgium
[d] The Resource Ecology Group, Department of Environmental Sciences, Wageningen University, Droevendaalsesteeg 3a, 6708PB Wageningen, The Netherlands

HIGHLIGHTS

- Innate immunity of wild great tits along a metal pollution gradient was studied.
- Metals were already present in the blood of 14-day old nestlings.
- Strongest effects of pollution were observed for lysis.
- The effects of metals on innate immunity were only detected at the level.
- This likely relates to high heterogeneity in exposure along the pollution gradient.

ARTICLE INFO

Article history:
Received 12 September 2014
Received in revised form 28 November 2014
Accepted 28 November 2014
Available online xxxx

Editor: F. Riget

Keywords:
Constitutive innate immunity
Nestlings
Natural antibodies
Complement
Nitric oxide
Acute phase proteins

ABSTRACT

Excessive deposition of metals in the environment is a well-known example of pollution worldwide. Chronic exposure of organisms to metals can have a detrimental effect on reproduction, behavior, health and survival, due to the negative effects on components of the immune system. However, little is known about the effects of chronic sublethal metal exposure on immunity, especially for wildlife. In our study, we examined the constitutive innate immunity of great tit (*Parus major*) nestlings ($N = 234$) living in four populations along a metal pollution gradient. For each nestling, we determined the individual metal concentrations (lead, cadmium, arsenic) present in the red blood cells and measured four different innate immune parameters (agglutination, lysis, haptoglobin concentrations and nitric oxide concentrations) to investigate the relationship between metal exposure and immunological condition. While we found significant differences in endogenous metal concentrations among populations with the highest concentrations closest to the pollution source, we did not observe corresponding patterns in our immune measures. However, when evaluating relationships between metal concentrations and immune parameters at the individual level, we found negative effects of lead and, to a lesser extent, arsenic and cadmium on lysis. In addition, high arsenic concentrations appear to elicit inflammation, as reflected by elevated haptoglobin concentrations. Thus despite the lack of a geographic association between pollution and immunity, this type of association was present at the individual level at a very early life stage. The high variation in metal concentrations and immune measures observed within populations indicates a high level of heterogeneity along an existing pollution gradient. Interestingly, we also found substantial within nest variation, for which the sources remain unclear, and which highlights the need of an individual-based approach.

1. Introduction

Large amounts of diverse chemical compounds are introduced into the environment via human activities, posing a great risk for wildlife. An important source of contamination affecting many areas around the world is the excessive deposition of metals. They are released into the environment via a range of human activities such as mining, metallurgy, vehicular traffic and pesticide use, but also via natural phenomena such as plate tectonics, forest fires and natural erosion (Bichet et al., 2013; Burger, 2008; Pagliara and Stabili, 2012). Metals contaminate the

---

* Corresponding author at: Department of Biology — Ethology, University of Antwerp, Campus Drie Eiken C1.10, Universiteitsplein 1, 2610 Antwerp (Wilrijk), Belgium.
E-mail addresses: anke.vermeulen@uantwerpen.be (A. Vermeulen), wendt.mueller@uantwerpen.be (W. Müller), k.d.matson@rug.nl (K.D. Matson), b.i.tieleman@rug.nl (B. Irene Tieleman), lieven.bervoets@uantwerpen.be (L. Bervoets), marcel.eens@uantwerpen.be (M. Eens).

http://dx.doi.org/10.1016/j.scitotenv.2014.11.095
0048-9697/

air, soil and water, enter the food chain, accumulate in organisms (Dedourge-Geffard et al., 2009; Jaspers et al., 2004; Nolet et al., 1994; Roggeman et al., 2013) and can have detrimental effects on among others condition, reproduction, breeding performance and survival in a large range of animals (Brasso and Cristol, 2008; Eeva et al., 2005a, 2012; Fair et al., 2003; Gorissen et al., 2005; Janssens et al., 2003b, 2003c; Moron et al., 2014; Pedersen and Saether, 1999; Scheuhammer, 1987; Witeska et al., 2014). In addition, metals can also have genotoxic effects such as for example DNA damage in wildlife (Pastor et al., 2001; Sebbio et al., 2014).

These negative effects may be mediated via the effects of metals on immune function (Borowska and Pyza, 2011; Das et al., 2008; Day et al., 2007; McMurry et al., 1995; Pagliara and Stabili, 2012; Tersago et al., 2004). As the immune system is critical for defense against pathogens and other threats, immunosuppressive effects of contaminant exposure probably reduce fitness (Norris and Evans, 2000). In the case of birds, immunotoxic effects of metal pollution have been demonstrated, with the most convincing evidence originating from studies under controlled laboratory conditions or experimental feeding studies. These studies showed a suppression of total antibody production, a decrease in lymphocyte proliferation, a decline in circulating white blood cells and a suppression of natural, humoral and cell-mediated immune responses (Kenow et al., 2007; Lewis et al., 2013; Rocke and Samuel, 1991; Trust et al., 1990; Vodela et al., 1997; Youssef et al., 1996). Fewer studies have investigated the immunological effects of free-living birds in their natural environment where the pollution is in fact occurring. However, the results of these studies are not consistent since some find negative effects of metal pollution on cell-mediated or humoral immunity, while others find no effect on cell-mediated immunity or immunocompetence in general (Baos et al., 2006; Elbert and Anderson, 1998; Fair et al., 2003; Hawley et al., 2009; Snoeijs et al., 2004; Wayland et al., 2003). Why studies under natural conditions tend to differ remains unclear. It may relate to a higher variance, e.g. in contaminant concentrations. In particular since high doses of metals are generally toxic to most organisms, while in some cases low doses may favor biological responses (= hormesis) (Bartlett and Smith, 2003; Eeva et al., 2005a; Nain and Smits, 2011). Furthermore, the effect that a contaminant will have on organisms also depends on the time course of exposure. Birds that are chronically exposed to sublethal metal contamination will probably experience immunological effects (Snoeijs et al., 2004), potentially due to accumulation (Yu et al., 2011). Finally, not only the duration but also the timing of the exposure is relevant. Early development is a particularly critical and relevant period, where any effect may have long-lasting consequences (reviewed in Lindstrom, 1999). Potentially toxic concentrations of total arsenic (As), cadmium (Cd) and lead (Pb) can indeed already be found in the excrement of 15-day old nestlings (Janssens et al., 2003a) and in eggshells and egg content (Dauwe et al., 1999), indicating early life exposure. Nestlings are at a vulnerable developmental stage as their immune system continues to develop while they grow (Fair and Ricklefs, 2002). While the effects of metal pollution during early development are expected to broadly affect an organism's physiology, quantifying immunological effects can be methodologically and logistically challenging (Horrocks, 2011; Matson et al., 2005, 2012).

The central goal of this study was to investigate the relationship between metal exposure and immunological condition in great tit (*Parus major*) nestlings living along a pollution gradient that related to distance from a smelter. We assessed several aspects of innate immune function, which generally is non-specific and serves as an initial line of defense against invading pathogens. Natural antibodies (NAbs) and complement activity are two interrelated non-cellular components of innate immunity. Present in immunologically naïve individuals, NAbs broadly recognize and bind to antigens, a process which can result in activation of the complement cascade, which ends with the lysis of foreign cells (Boes, 2000; Matson et al., 2005; Murphy et al., 2012; Ochsenbein and Zinkernagel, 2000). Another aspect of innate immunity are acute phase proteins (APPs). APPs are typically synthesized by hepatocytes in response to cytokines released by macrophages in the presence of bacteria (Coon et al., 2011; Cray et al., 2009; Murphy et al., 2012; Owen-Ashley and Wingfield, 2007). APPs have several antimicrobial functions, such as activating the complement cascade and opsonizing bacteria (Murphy et al., 2012). The APP haptoglobin (Hp) circulates in the blood at low concentrations that rise significantly in response to an acute infection, trauma or inflammation (Cray et al., 2009; Matson et al., 2012; Murata et al., 2004; Quaye, 2008). Nitric oxide (NO) is a multifunctional signaling molecule, which acts as a vasodilator, neurotransmitter and a modulator of inflammatory processes. Assessing nitric oxide can provide useful information on individual variation in work load, physiological condition and health state (Bichet et al., 2013; Bourgeon et al., 2007; Sild and Horak, 2009). We focused on nestlings as they enable investigation of the impacts of pollution early in life, which, as pointed out above, is a life history stage where any change to the developmental trajectory may have significant fitness consequences. We first explored these relationships among different populations along a pollution gradient and we expected to find negative relationships between contamination and innate immune parameters. But given the possibility of small-scale variation in contamination (Fritsch et al., 2011), we also expect that this small-scale variation is reflected in our immune measures necessitating a refined approach. Thus, we additionally studied whether the immune parameters vary with metal concentrations on the individual level. The latter has, as yet, rarely been taken into account.

2. Material and methods

2.1. Study sites

Our study was conducted in four different great tit populations in a well-established pollution gradient near a non-ferrous smelter south of Antwerp (Hoboken), Belgium. Common pollutants in this area are lead, cadmium and arsenic (Table 1) (Dauwe et al., 1999; Geens et al., 2010; Janssens et al., 2001; VMM, 2011). Each study site is located at a different distance east of the smelter with Umicore located near the smelter 0–350 m, Fort 8 located 400–600 m to the east of the smelter, Fort 7 located 2500 m to the east and finally Fort 4 located 8500 m eastwards of the factory (Fig. 1). All study sites have a similar habitat type, which can be classified as deciduous park area (Janssens et al., 2001). Both traffic intensity and urbanization levels are high but comparable among all study sites. Great tits living at these different sites breed in nest boxes with approximately 30 to 60 nest boxes per study site and similar nest box densities.

2.2. Data sampling

During the breeding season of 2012 (March–May), we checked nest boxes every other day to determine the laying date, clutch size, start of incubation and hatch day. When nestlings were 14 days old (hatch day = day 1), a blood sample (±150 μL) was obtained by puncturing the brachial vein with a 27 gauge needle and collecting the blood with a Microvette CB 300 lithium-heparin tube (Sarstedt). The collected

Table 1
Range of mean annual metal concentrations in atmospheric particulate matter (PM_{10} ng/m^3) with their reference values as set by the European guide line (EU) and mean annual metal depositions (μg/m^2 day) with their reference values as set by the Flemish regulations concerning environmental permits (VLAREM II) in the area surrounding the smelter (information taken from VMM, 2011).

	PM_{10} (ng/m^3)	Reference value EU (ng/m^3)		Deposition (μg/m^2 day)	Reference value VLAREM II (μg/m^2 day)
Pb	80–258	500	Pb	314	250
Cd	1.3–2.9	5	Cd	3.9	20
As	9.6–44	6	As	24	No reference value set