Polar Biol
DOI 10.1007/s00300-006-0175-9

ORIGINAL PAPER

Geographic variation in the immunoglobulin levels in pygoscelid penguins

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Received: 3 April 2006 / Revised: 25 May 2006 / Accepted: 5 June 2006

Abstract Antarctic organisms, including penguins, are susceptible to parasites and pathogens. Effects of infestation could differ in different locations along a geographical gradient from north to south consistent with conditions that affect the prevalence and virulence of parasites and pathogens. The immune system, including immunoglobulins as the main component of the humoral immune response, is the major way by which organisms confront infestation. We investigated the variation in immunoglobulin levels in three species of antarctic penguins (*Pygoscelis antarctica*, *Pygoscelis papua*, and *Pygoscelis adeliae*) along a geographical gradient from King George Island (62°15′S) to Avian Island (67°46′S). We found that immunoglobulin levels increased northwards in all the three species. This could indicate a higher impact of parasites and/or pathogens relative to the existing gradient in temperatures along this coast. Changing temperatures, consistent with global climate change, could be altering the ecology of parasite or pathogen infestation within the biota of northern Antarctica. We have also found marginal differences in immunoglobulin levels between sexes in both chinstrap and gentoo penguins.

Introduction

Ecological studies during the last 10 years have emphasised the importance of parasites and diseases as selection pressures affecting organismal fitness in terms of condition, survival prospects, or reproductive success (see Clayton and Moore 1997). The immune system comprises the main way by which hosts defend against parasites and pathogens. In birds, this system works in two ways: humoral and cell-mediated immunity (Wakelin and Apanius 1997) which belong to the acquire immune response. Studies on the cell-mediated response have increased mainly due to the use of the phytohaemagglutinin technique (Smits et al. 1999; Merino et al. 1999; Barbosa and Moreno 2004). On the other hand, studies on the humoral response have been based on the measure of the level of total antibodies responding to injection with sheep red blood cells (e.g. Ots et al. 2001) or the response against specific non-pathogenic antigens (Raberg et al. 2000). The present paper focuses on the variation of immunoglobulin levels that is used as a monitoring method to assess humoral immunity. Several studies have been carried out recently estimating the circulating level of total non-specific gamma globulins (IgY in birds) (Ots and Horak 1998; Johnsen and Zuk 1999; Szep and Møller 1999; Morales et al. 2004). Immunoglobulins are highly variable proteins synthesised by the B lymphocytes of

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the immune system and the plasma cells derived from them. They are components of the antibody response against pathogens such as viruses, bacteria, and both endo- and ectoparasites (Roitt et al. 1996; Wikel 1996; Pastoret et al. 1998). There are several reasons for studying total concentrations of immunoglobulins: (1) it does not require prior capture for immunisation, (2) it gives valuable insights into the health of birds in natural conditions (Gustaffson et al. 1994), and (3) it has been used as a measure of immunocompetence (Johnsen and Zuk 1999; Saino et al. 2001; Morales et al. 2004). This technique can reflect both a higher parasitic load and/or a higher immunocompetence.

Prevalence and intensity of parasites and pathogens are known to vary spatially (Bennett et al. 1995; Merila et al. 1995; Sol et al. 2000) and, therefore, immunity is expected to vary geographically to the same extent (Dupas and Boscaro 1999). However, very few studies have quantified the extent of this variation, and to our knowledge, no studies on this topic have been carried out in Antarctica.

Antarctic organisms including penguins are susceptible to parasites and pathogens (see Kerry et al. 1999), including ectoparasites such as ticks, fleas, or biting lice; endoparasites such as nematodes and cestodes; and several diseases produced by both viruses and bacteria. Although only a few events of mass mortality have been reported (Kerry et al. 1999), the effects of parasites and pathogens on the fitness of Antarctic penguins can be important as has been shown in South American penguin species (Potti et al. 2002). Under a climate change scenario, it could be hypothesised that an increase in temperature would produce a change in the range, abundance, and virulence of parasites (Lindgren 1998; Lindgren et al. 2000; Sutherst 2001). In fact, climatic variation could have direct effects on immune defence (Møller 2002; Møller and Erritzoe 2003). Moreover, the Antarctic Peninsula has suffered from faster and higher temperature increase than anywhere else in the world (King et al. 2003).

In this paper, we investigate the variation in natural immunoglobulin plasma levels in three species of Antarctic penguins in different locations from north (62°15′S) to south (67°46′S) along the west coast of the Antarctic Peninsula. We want to determine whether geographical variation in immunity exists and to establish a baseline for future comparisons.

Materials and methods

Three species of pygoscelid penguins were studied: Chinstrap penguin (*Pygoscelis antarctica*), gentoo penguin (*Pygoscelis papua*), and Adélie penguin (*Pygoscelis adeliae*). The geographic ranges of the gentoo, chinstrap, and Adélie penguins are 46–65°S, 56–65°S, and 54–77°S, respectively. Therefore, the geographic range studied constitutes the intermediate part of the Adélie penguin distribution and the southern part of the chinstrap and gentoo penguin distributions. Reported parasites in these species include intestinal helminths (Sladen 1954; Kerry et al. 1999; J. Valencia, pers. com.). No blood parasites have been recorded in these species in Antarctic (Merino et al. 1997; Jones and Shellam 1999).

During January and February 2003, we visited several penguin breeding localities along the Antarctic Peninsula (Table 1). Adult penguins were captured on the beach in order to minimise disturbance in the breeding colonies. In order to make comparisons among different localities, adults were chosen instead of chicks as the latter ranged widely in degree of development. We sampled the penguin populations when the chicks were in guard phase to avoid the likely effects of variation related to the breeding period. From each individual, we measured body mass and took a blood sample from the foot vein with a heparinised capillary immediately after capture. This sample was later centrifuged at 12,000 rpm during 10 min to separate plasma from red blood cells. We then ob-

Table 1 Localities, species studied, and sample sizes

Localities		Species	Sample size	Region
Point Thomas (King George I.)	62°10′S, 58°29′W	*Pygoscelis adeliae*	25 (9F, 4M)	1
		Pygoscelis papua	10 (7F, 3M)	
Miers Bluff (Livingston I.)	62°43′S, 60°26′W	*Pygoscelis antarctica*	25 (6F, 17M)	1
Baily Head (Deception I.)	62°58′S, 60°30′W	*Pygoscelis antarctica*	25 (10F, 13M)	1
George Point (Ronge I.)	64°40′S, 60°40′W)	*Pygoscelis antarctica*	25 (10F, 10M)	2
		Pygoscelis papua	25 (11F, 9M)	
Torgersen I.	64°46′S, 64°04′W	*Pygoscelis adeliae*	25 (5F, 18M)	2
Avian I.	67°46′S, 68°43′W	*Pygoscelis adeliae*	25 (5F, 15M)	3

Mean annual temperature for region 1 = –2.4°C, region 2 = –4.0°C, and region 3 = –4.6°C (data taken from Turner et al. 2004)

F females, *M* males

tained the haematocrit as the relative percentage of red blood cells in the total volume of blood (red blood cells volume + plasma volume). To measure circulating levels of total IgY, the plasma fraction was analysed by means of a direct ELISA using peroxidase-conjugated anti-chicken IgY antibodies (Sigma, St Louis, MO, USA, A-9046). The linear range of the sigmoidal curve for this antibody–antigen response, as well as the optimal serum dilution (1/50,000), had been previously determined (Martínez et al. 2003). Absorbances were measured using a plate spectrophotometer at $\lambda = 405$ nm. For details see Martínez et al. (2003).

To account for sex variation within each species, we sexed the individuals by means of molecular markers (Ellegren 1996). In the case of the Adélie penguin, we used a PCR-RFLP method (Boutette et al. 2002).

For statistical analyses, we grouped localities within the same geographical degree (see Table 1). Data were analysed with generalised linear models (GLM), with region and sex as factors, and body mass and haematocrit as covariates. A different GLM was performed to account for differences among the species using the same covariates. We used backward stepwise selection procedures to determine which variables accounted best for the variation in the dependent variable. The criterion to remove a variable was set at $P = 0.05$.

Results

Results of the analyses within species showed that geographical location significantly explained the variation found in immunoglobulin level in the three species. Sex showed marginally significant differences in immunoglobulin level in chinstrap (females = 0.90 ± 0.03 SE, higher than males = 0.84 ± 0.03 SE) and gentoo penguin (males = 1.33 ± 0.07 SE, higher than females = 1.18 ± 0.05 SE). Northern populations had higher immunoglobulin levels than southern populations (Adélie penguin: region, $F_{2,62} = 2.99$, $P = 0.05$, Fig. 1a; sex, $F_{1,61} = 1.11$, $P = 0.29$; chinstrap penguin: region, $F_{1,56} = 16.39$, $P < 0.001$, Fig. 1b; sex, $F_{1,55} = 3.36$, $P = 0.07$; gentoo penguin: region, $F_{1,20} = 12.97$, $P < 0.05$, Fig. 1c; sex, $F_{1,19} = 3.59$, $P < 0.07$). Body mass and haematocrit did not show any significant results in any of the three species considered.

We found differences among the three species of pygoscelid penguins ($F_{2,152} = 34.77$, $P < 0.001$). Gentoo penguin differed from the other two species showing the highest level of immunoglobulins (Fig. 2). The covariates did not show any significant result.

Discussion

Geographic variation in immune parameters in animals has been addressed mainly in *Drosophila* and its parasitoids (Dupas and Boscaro 1999; Kraaijeveld and Godfray 1999; Fellowes and Godfray 2000); very few studies have been carried out on other organisms including birds (Grasman 2002). To our knowledge, there are no studies on geographic variation in the

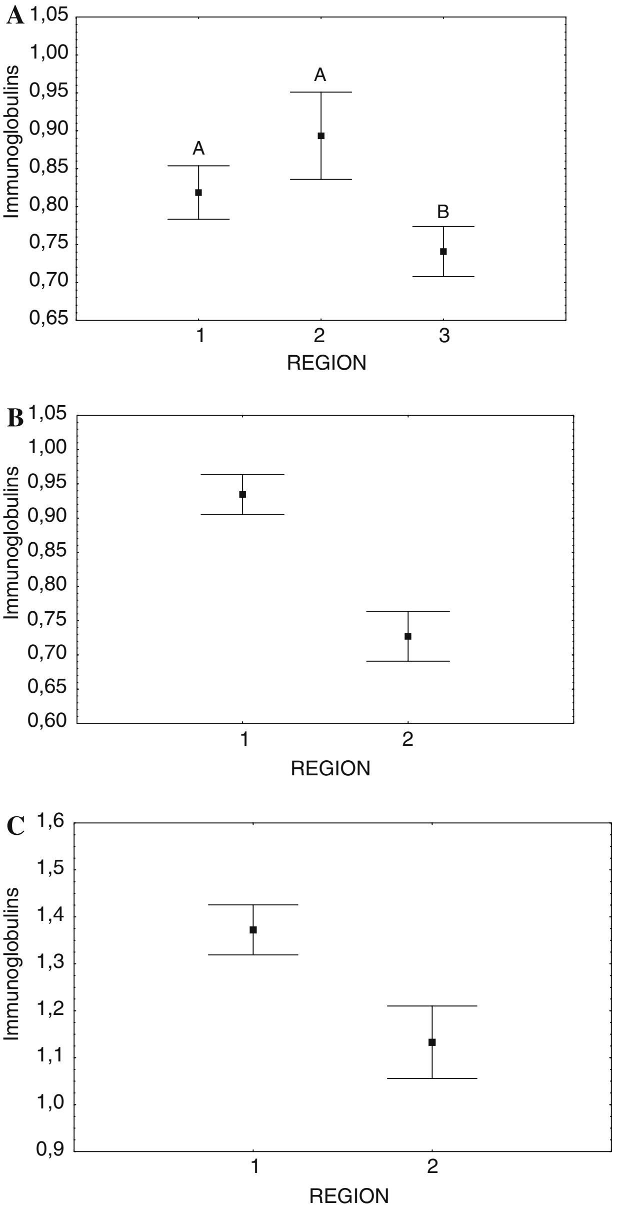

Fig. 1 **a** Geographical differences in immunoglobulin level in the Adélie penguin (*Pygoscelis adeliae*). *Letters* denote differences between groups by a posteriori Scheffe test. **b** Geographical differences in immunoglobulin level in the chinstrap penguin (*Pygoscelis antarctica*). **c** Geographical differences in immunoglobulin level in the gentoo penguin (*Pygoscelis papua*)

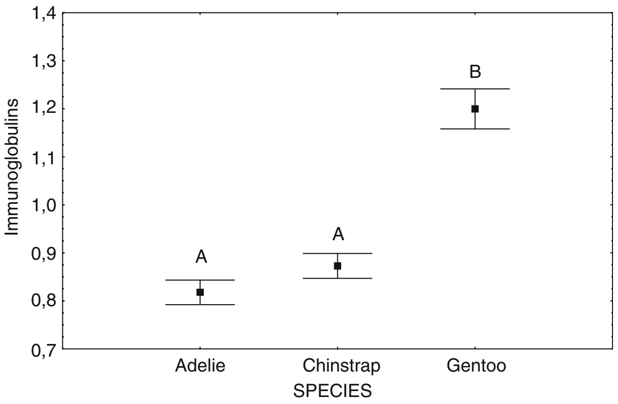

Fig. 2 Differences in the immunoglobulin levels among the three species of penguins. *Letters* denote differences between groups by a posteriori Scheffe test

immune parameters for organisms living in Antarctica (Moreno et al. 1998; Meloni et al. 2000) except for humans (Olson 2002 and references therein).

Our results show, therefore, for the first time, a geographic variation in immune parameters in wild organisms such as penguins on this continent. We found that immunoglobulin levels increase northwards in the three species of penguins studied. These results were not diminished by body mass and haematocrit. The high level of immunoglobulins in the northern populations of penguins could indicate a higher impact of parasites and/or pathogens in the north than in the south along the Antarctic Peninsula. We only know that blood parasites are not present in the localities studied (unpublished data; Merino et al. 1997), but there could a geographical variation of intestinal parasites and/or pathogens such as bacteria. Warmer conditions in northern localities in Antarctic Peninsula (see Table 1, Turner et al. 2004) could favour higher prevalence or virulence of parasites or pathogens.

Higher immunoglobulin levels in northern populations also could be due to the greater impact of human activity in this region. Human effects can be of two kinds. First, the accidental introduction of pathogenic organisms (Gardner et al. 1997; Kerry et al. 1999; Gauthier-Clerc et al. 2002) can produce infections on penguins living in such areas. Nevertheless, transmission and dispersion of diseases by birds themselves from contact between sub-Antarctic and Antarctic penguin populations foraging together in the Antarctic pack ice during winter cannot be excluded (Gauthier-Clerc et al. 2002). This scenario could be more plausible in northern populations of gentoo penguin and chinstrap penguin as these populations seem to travel to the north during winter (Williams 1995).

A second effect of human activities could be an increase of contamination and/or direct stress on the penguin populations through visits by tourists. High levels of contamination have shown immunosuppressive effects both in captivity (Redig et al. 1991) and in the wild (Snoeijs et al. 2004). On the other hand, higher levels of stress may produce a reduction of the immune response as well (Raberg et al. 1998). Therefore, this kind of human impact would produce a decrease in immune levels in northern populations instead of the high level of humoral immunity we found. Thus, these factors can be excluded as explanation of geographic differences among populations, at least in the localities we sampled. However, sampling of penguin colonies with a high pressure of tourism and studies on geographic variation of contamination levels should be carried out to investigate these potential effects.

Alternatively, a lower immunoglobulin plasma concentration in the south could be explained if birds in the south faced different energetic constraints (i.e. temperature or food resources) leading to less energy for immunity. As far as we know, there are yet to be published any studies on the geographic variation of foraging energetics in penguins. Therefore, this possibility remains to be tested. With respect to thermoregulation, although differences in temperature and humidity among the different regions considered could affect in some way the energetic demands, this should be important in winter but not in summer when the samples were taken because temperatures at this time usually are close to the thermoneutral zone for these species [–10°C for Adélie penguin (Chapell and Souza 1988) and 5–10°C for gentoo penguin (Taylor 1985)]. Anyway, the relationship between temperature and the immune function in these species remains to be studied.

We sampled the penguin populations when chicks were in guard phase precluding the likely effect of variation related to breeding period, as it has been found in barn swallows (Saino et al. 2001). The female of this species showed a variation in immunoglobulin levels during the breeding cycle being higher just before laying. Therefore, differences among populations could be explained by differences in breeding phenology. In fact, a geographical gradient has been described in the laying date of penguins from north to south in Antarctica (Williams 1995). However, Morales et al. (2004) did not find differences in immunoglobulin levels between incubation and the end of the nestling phase for pied flycatcher (*Ficedula hypoleuca*) females. Considering that we took the samples during this same phase, we can exclude this alternative explanation.

We also found marginally significant sexual differences in the immunoglobulin levels in both chinstrap and gentoo penguin although in the opposite direction. In chinstrap penguins, the females showed higher levels but in gentoo penguins, they were higher in males. Usually females show higher immunity than males in both domestic (Terres et al. 1968; Grossman 1984) and wild animals (Møller et al. 1998; Barbosa and Moreno 2002), including penguins (Moreno et al. 2001), mainly due to the immunosuppressive effect of androgens like testosterone. Although chinstrap penguin results are in agreement with this general pattern, no sex differences have been reported in cell-mediated immune response (Moreno et al. 1998), while no other studies have explored immunity in gentoo penguins. Nevertheless, some authors have found a higher level of immunity in humoral response in males of zebra finches (McGraw and Ardia 2005) related to the presence of colour traits and the immunostimulatory role of carotenoids in the colourful sex, that is, the males. Gentoo penguin was the only one of the three species we studied that showed a colour trait, the red bill. Although no studies have been carried out on this species whether this is a caroteinoid-mediated trait, colour bills in other species have been proven to be the case (Blount et al. 2003). This could explain the finding that males have higher humoral immunity level than females. Therefore, more research is needed on the relationship between these sorts of factors and immunity in Antarctic penguins.

Finally, although comparisons of immunoglobulin level among species using direct ELISA method should be taken with caution due to likely species-specific differences in anti-chicken affinity for the different IgY of each species (Martínez et al. 2003), we found differences in immunoglobulin levels among species, showing the gentoo penguin the highest level. Although there is little information about how different aspects of immune function correlate across species (Tella et al. 2002), species-specific differences in immunity have been shown to be due to differences in exposition to parasites and/or pathogens (Møller and Erritzoe 2002) in relation to differences in habitat use (Møller 1998), coloniality (Møller et al. 2001), dispersion (Møller and Erritzoe 2001), migration (Møller and Erritzoe 1998), sexual selection intensity (Nunn 2002), and mating system (Nunn et al. 2000) among others. Some of these factors could explain the differences observed in immunity among the three species of pygoscelids studied in the present work; however, there are no studies in which these likely differences have been addressed. Therefore, more investigation is needed to unveil the causes of specific differences in the level of immunoglobulins obtained.

Acknowledgments This study has been funded by the Acción Especial project REN2001-5004/ANT of the Spanish Ministry of Education and Science. The project CGL2004-01348/ANT supported AB while the paper was written. We very much appreciated the hospitality and logistic support of the Spanish Antarctic Bases “Juan Carlos I,” “Gabriel de Castilla” and the Spanish Polar Ship “Hesperides” and specially the Spanish Polar Ship “Las Palmas” which provided us the logistic support and transport to the localities. We thank Elena Arriero and Rafa Barrientos for laboratory assistance with molecular sexing. We also thank two anonymous referees for their comments that improved early versions of the manuscript.

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