J. Ornithol. 143, 351–355 (2002)

ISSN 0021-8375

Annual variation of immune condition in the Hooded Crow (*Corvus corone cornix*)

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Summary

Little is known about indices of health condition in free-living populations and particularly about the presence of significant fluctuations of these indices between years. We assessed blood and immunological condition in wild Hooded Crows (*Corvus corone cornix*) in NW Italy for three years (1997–1999). Crows did not show any year-to-year difference in erythrocyte sedimentation rate, leukocyte abundance, and heterophyl/lymphocyte ratio. In contrast, we observed significant annual differences in albumin and immunoglobulin values. The albumin/immunoglobulin ratio was lower in 1998, a year when the size of two immunocompetence organs (bursa of Fabricius and spleen) was also smallest. Neither population density nor climate were likely to affect the observed variation of immune condition, annual censuses not revealing any noticeable density variation during the study period, and rainfall and mean temperatures being similar. The results show that in natural populations between-year variation of immune condition may exist, and that in our study, species immunoglobulin assays were more effective than leukocyte counts to detect them.

Keywords: erythrocyte sedimentation, heterophyl/lymphocyte ratio, immunoglobulin, immunocompetence.

Zusammenfassung

Jährliche Variation immunologischer Parameter bei Nebelkrähen (*Corvus corone cornix*)

Über Parameter, die den Gesundheitszustand freilebender Vögel indizieren, ist nur wenig bekannt. Wir haben immunologische Parameter von Nebelkrähen (*Corvus corone cornix*) in NW Italien über drei Jahre (1997–1999) verfolgt. Die Krähen zeigten keine jährliche Variation in der Erytrozytensenkungsgeschwindigkeit, in der Zahl der Leukozyten oder im Heterophilen/Lymphozyten-Verhältnis. Signifikante jährliche Schwankungen gab es jedoch in den Albumin- und Immunoglobulinwerten. Das Verhältnis von Albumin zu Immunoglobulinen war am geringsten in 1998, in dem Jahr in dem die Größe zweier immunkompetenter Organe (bursa Fabricius und Milz) am kleinsten war. Weder die Populationsdichte noch das Klima scheinen für diese Schwankungen verantwortlich zu sein, da beide während des Beobachtungszeitraum relativ konstant waren. Unsere Ergebnisse zeigen, dass der Immunstatus natürlicher Populationen jährlichen Schwankungen unterliegt, die nicht mit Leukozytenzählung wohl aber durch Quantifizierung der Immunoglobuline nachgewiesen werden können.

Introduction

The immune system plays a decisive role in defending an animal against attack by pathogens and parasites. Environmental stresses, such as sudden variation in weather, food shortages or overcrowding, cause alterations in host immunocompetence, and all these factors ultimately influence the fluctuations of animal populations (Lockmiller & Dabbert 1993). The demonstration that external factors modulate immune responsiveness is based mainly on laboratory studies of poultry or semi-domesticated animals (Dabbert et al. 1997, Gross & Siegel 1983).

Among mammals, it has been proposed that poor weather in winter enhances immune responsiveness in wild populations of Prairie Voles (*Microtus ochrogaster*), suggesting a circannual cycle for immunocompetence (Sinclair & Lochmiller 2000). In this species, a high population density enhanced immunocompetence, while in female Bank Voles (*Clethrionomys glareolus*) the opposite was found (Saino et al. 2000). Although immunological ecology relies mainly on ornithological research (Sheldon & Verhulst 1998), few studies in birds have compared the immune response in different years, nor have differences in blood parameters among individuals living in different localities been assessed.

In the Hooded Crow (*Corvus corone cornix*), we found annual variation in body mass and immunocompetence organ sizes (spleen and bursa of Fabricius) in two localities (Acquarone et al. 2001). In this study, we monitored blood parameters for three years in the same localities in NW Italy. The aim was to verify if year-to-year differences found in body mass and immunocompetence organ sizes correspond to differences in blood parameters. In order to understand why those variations occurred, in a preliminary investigation we explored the corresponding variation of yearly climatic conditions and population density. The data was to provide us with new insights into the flexibility of the immune system of birds.

Methods

The study was carried out at two sites in the province of Alessandria, NW Italy, in the springs of 1997–1999. A total of 248 individuals from Castellazzo and 162 from Novi were caught in April and May by Larsen traps as part of the provincial administration's agricultural pest management program. Sex was determined by gonadal examination, and age by examining the upper mandible and plumage colours (Svensson 1992). A few hours after trapping (1–3 hours), we measured each individual's mass and collected smears and blood samples. The birds were then exterminated by authorized personnel of the provincial administration according to the pest management procedure. We counted leukocytes and red blood cells in blood smears stained by the May-Grunwald-Geimsa method. Blood smears were scanned at 630x magnification following standard routines. In each microscopic field, we counted red blood cells and leukocytes classified as lymphocytes, monocytes, eosinophils, heterophils and basophils. In each smear, we counted 100 leukocytes and the corresponding red blood cells. We then calculated the relative frequency of each family of leukocytes with respect to the total leukocyte population (relative counts) and the number of leukocytes of each family per 10,000 red blood cells (absolute counts). This method provides significantly repeatable relative and absolute leukocyte counts (Saino et al., 1995). Absolute values were log10 transformed for statistical analysis.

To measure immunoglobulins, we performed a densitometric analysis after separation of serum proteins on gel. For each animal, about 1 ml of blood was collected in Sigma ACD after wing vein puncture, and immediately stored in a bag at 4 °C to be transported to the laboratory. Blood samples were centrifuged for 10 min at 4000 rpm. Five ml of serum was diluted 1:2.5 in Tris Barbital buffer (pH 8.8). Ten ml of the diluted sample was applied to agarose gel according to the standard Hydrasys kit procedure (Sebia, Issy-les-Moulineaux, France). Electrophoresis was conducted at constant voltage (120 V), 20 °C, for 7 min. The gels were then air-dried and stained according to kit instructions. Densitometric analysis was performed with a computer image analysis procedure run by the Hydrasys

System programme. The relative titre of Ig (albumin, α-, β- and γ-globulins) was expressed as the ratio between the area of the densitometric profile corresponding to the immunoglobulin region and the total area of the densitometric profile. Values were arcsine square root transformed for statistical analysis.

To measure the sedimentation rate, 70 microL blood samples in heparinised haematocrit capillary tubes were placed in a vertical position for 4 h in a refrigerated container (4 °C). According to Saino & Møller (1996), the sedimentation rate (proportion of blood forming a sediment per hour) was expressed as: (volume of the part of the capillary not occupied by blood cells) / (blood volume in the capillary) × 0.25.

In the three study years, we utilized the line transects method to assess the abundance of Hooded Crows in the area (details in Acquarone et al. 2001b). Crow density showed only slight variation during the study period; thus we did not perform any analysis of the influence of density on blood parameters. Meteorological data were obtained from the station at Casale (Alessandria). We compared the climatic conditions in different years by analysing the temperature and rainfall values for April and May, the months in which the crows were caught.

In the Hooded Crow, males are significantly larger than females and adults are larger than subadults. Therefore, we conducted a multivariate analysis of variance (MANOVA) with blood parameters as response variables, and sex, age, site and year as factors (Wilkinson 1998).

Results

Mean temperature during the capture period (from April to May) did not differ between years ($F_{2,180} = 1.28$, $p = 0.28$) nor was there a difference in rainfall in the three years ($F_{2,180} = 1.48$, $p = 0.23$).

Mean values of erythrocyte sedimentation rate, leukocyte abundance, and heterophyl/lymphocyte ratio were not influenced by gender, or age, site and year (MANOVA statistic Table 1).

Albumin and immunoglobulin values were available only for the site of Castellazzo. A MANOVA revealed significant year-to-year differences in albumin and γ-globulin values, while there were no gender or age related differences (Table 2). Because of the high value of γ-globulin, the albumin/immunoglobulin ratio was lowest in 1998.

Discussion

In this study we monitored for three years the values of blood parameters in free-living Hooded Crows. Mean values were in the expected range for passerine birds (Prinzinger & Misovic 1994, Merilä & Svensson 1995), and individuals were similar regardless of gender, age and site.

We found year-to-year fluctuations of immune plasma proteins (albumin and immunoglobulin), while there were no interannual variation in white blood cell counts (leukocyte abundance, heterophil/lymphocyte abundance and ratio) and in erythrocyte sedimentation rate. The albumin/immunoglobulin ratio was lowest in 1998, when a noticeable increase of immunoglobulin occurred. Elevation of immunoglobulin usually indicates activation of the immune system and is most often due to infection. Interestingly, in the same population, body mass and immunocompetence organ masses (spleen and bursa of Fabricius) were also at their lowest levels in 1998 (Acquarone et al. 2001). The avian spleen and the bursa of Fabricius are very important immune defence organs involved in the production of antibodies designed to combat a range of different pathogens and parasites. On the assumption that a larger bursa or spleen can produce a better immune defence than a smaller organ, in 1998 Hooded Crows probably suffered more critical ill-health conditions.

The proximate cause of unfavourable health conditions in 1998 remains undetected. In this study, neither density nor general climatic factors (temperature or rainfall) explained the fluctuations of blood parameters: there was no relevant variation of bird density in the study

Table 1. Multivariate analysis of variance MANOVA of haematological parameters in three years (1997 to 1999).
Tab. 1. Multivariate Analyse (MANOVA) hämatologischer Parameter von Nebelkrähen der Jahre 1997–1999.

		MANOVA independent variables							
		SEX		AGE		SITE		YEAR	
Dependent variable	mean ± se	F	P	F	P	F	P	F	P
Sedimentation rate	0.279 ± 0.007	0.08	ns	2.39	ns	0.10	ns	0.38	ns
Leukocytes[1]	126.9 ± 7.03	1.64	ns	0.22	ns	0.01	ns	0.33	ns
Heterophils (%)	18.6 ± 0.86	1.02	ns	0.76	ns	1.72	ns	0.33	ns
Lymphocytes (%)	67.5 ± 1.69	2.02	ns	0.04	ns	3.06	ns	0.63	ns
H/L ratio	0.30 ± 0.10	1.88	ns	0.20	ns	0.04	ns	1.48	ns
MANOVA Wilk's lambda		0.98	ns	0.98	ns	0.91	ns	0.96	ns

[1] Leukocytes abundance reported as N / 10000 red blood cells.

Table 2. Multivariate analysis of variance MANOVA of immune proteins in three years (1997 to 1999).
Tab. 2. Multivariate Analyse (MANOVA) von Immunproteinen von Nebelkrähen der Jahre 1997–1999.

		MANOVA independent variables					
			SEX		AGE		YEAR	
Dependent variable		mean ± se	F	P	F	P	F	P
Albumin	1997	33.8 ± 1.06	0.60	ns	0.06	ns	3.60	< 0.05
	1998	31.8 ± 1.06						
	1999	28.8 ± 1.02						
γ-globulin	1997	36.2 ± 1.03	3.25	ns	0.30	ns	29.3	< 0.001
	1998	41.6 ± 1.04						
	1999	31.1 ± 1.02						
MANOVA Wilk's lambda			0.94	ns	0.99	ns	0.39	< 0.01

period and temperatures and rainfall were similar in the three years.

Between the three study years, leukocyte abundance and the sedimentation rate did not vary. Leukocyte counts (Ots et al. 1998) and sedimentation rate (Merilä & Svensson 1995) have been reported to be useful indicators of stress in birds. The absence of variation in the sedimentation rate could be due to the fact that, in the Hooded Crow, only individuals with severe loss of weight show critical sedimentation rate values (Acquarone et al. 2002). In this light, we hypothesize that our crows never reached a dangerous pathological threshold in free-living conditions.

In conclusion, the existence of year-to-year variation of body condition (Bosch et al. 2000, Acquarone et al. 2001) and immune status (this study) suggest that a carefully designed study of health condition in birds should take into account interannual variation, and that in Hooded Crows the immunoglobulin assays

were more effective than leukocyte counts to detect them.

Acknowledgements

We thank M. Foco of the Alessandria hospital for immunoglobulin assay, N. Saino for advice on leukocyte counts, S. Ceratto, F. Silvano and the Alessandria Provincial Administration who allowed us to operate on their trapped Hooded Crows, and A. Scotti and S. Bisoffi of the Pioppicoltura Institute, who provided meteorological data. The study was supported by 40–60 % MURST grants.

References

Acquarone, C., Cucco, M. & Malacarne, G. (2001): Short-term effects on body condition and size of immunocompetent organs in the hooded crow. Ital. J. Zool. 68: 195–199.

Acquarone, C., Cucco, M. & Malacarne, G. (2002): Effects of food abundance and unpredictability on body condition and health parameters: experimental tests with the Hooded Crow. Ibis (in press).

Bosch, M., Oro, D., Cantos, F. J. & Zabala, M. (2000): Short-term effects of culling on the ecology and population dynamics of the yellow-legged gull. J. Appl. Ecol. 37: 369–385.

Dabbert, C. B., Lochmiller, R. L. & Teeter, R. G. (1997): Effects of acute thermal stress on the immune system of the northern bobwhite. Auk 114: 103–109.

Gross, W. B. & Siegel, H. S. (1983): Evaluation of the heterophil/lymphocytes ratio as a measure of stress in chicken. Avian Disease 27: 972–979.

Lochmiller, R. L. & Dabbert, C. B. (1993): Immunocompetence, environmental stress and the regulation of animal population. Comp. Biochem. Physiol. 1: 823–855.

Merilä, J. & Svensson, E. (1995): Fat reserves and health state in migrant Goldcrest *Regulus regulus*. Funct. Ecol. 9: 842–848.

Ots, I., Murumägi, A. & Hõrak, P. (1998): Haematological health state indices of reproducing Great Tits: methodology and sources of natural variation. Funct. Ecol. 12: 700–707.

Prinzinger, R. & Misovic, A. (1994): Vogelblut – eine allometrische Übersicht der Bestandteile. J. Ornithol. 135: 133–165.

Saino, N., Canova L., Fasola M. & Martinelli R., (2000): Reproduction and population density affect humoral immunity in bank voles under field experimental conditions. Oecologia 124: 358–366.

Saino, N. & Møller, A. P. (1996): Sexual ornamentation and immunocompetence in the barn swallow. Behav. Ecol. 7: 227–232.

Saino, N., Møller, A. P. & Bolzern, A. M. (1995): Testosterone effects on the immune system and parasite infestations in the barn swallow (*Hirundo rustica*): an experimental test of the immunocompetence hypothesis. Behav. Ecol. 6: 397–404.

Sheldon, B. C. & Verhulst, S. (1998): Ecological immunology: costly parasite defences and trade-offs in evolutionary ecology. Tree 11: 317–321.

Sinclair, J. A. & Lochmiller, R. L. (2000): The winter immunoenhancement hypothesis: associations among immunity, density, and survival in prairie vole (*Microtus ochrogaster*) populations. Can. J. Zool. 78: 254–264.

Svensson, L. (1992): Identification guide to European passerines. (4th ed.). Norfolk UK.

Wilkinson, L. (1998): Systat (ver. 8.0). Chicago.

Accepted: 26. November 2001