Immune Response to Newcastle Disease Virus Vaccination in a Wild Passerine

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ABSTRACT: We studied the immune response of wild House Sparrows (*Passer domesticus*) experimentally challenged with different doses of inactivated Newcastle disease virus (NDV) vaccine. We evaluated within-individual cell-mediated and humoral responses in birds kept in outdoor aviaries, over a 6-wk period. Nonbreeding adult House Sparrows developed a significant humoral response to NDV experimental vaccination within 1 wk postchallenge, as measured by hemagglutination inhibition assay; values increased until week 4 and persisted until week 6. Differences among treatments appeared by week 1, with individuals challenged with the highest dose (0.2 mL) eliciting a higher humoral response than the rest ($n=18$). By week 4, all individuals vaccinated displayed an increased humoral response, with individuals challenged with the highest dose remaining significantly above responses of individuals vaccinated with the middle dose (0.1 mL, $n=14$), but not the lowest dose (0.05 mL, $n=15$). The middle and lowest dose responded similarly and significantly different from controls ($n=23$). Differences persisted through week 6 postchallenge. Cell-mediated responses were independent of the experimental treatment. All individuals experienced a rise in granulocyte concentration, whereas lymphocyte and monocyte concentrations decreased, most likely as a result of captivity. Adult wild House Sparrows immunochallenged with inactivated NDV vaccine developed a specific humoral response, highlighting the utility of this technique in immunologic and evolutionary ecology studies in wild birds.

Key words: Cell-mediated response, flow cytometry, hemagglutination inhibition, House Sparrow, humoral response, *Passerdomesticus* .

Because parasites and pathogens can have a critical effect on individual fitness, protection against disease is of major importance. However, pathogen avoidance and investment in immune function often interact with other vital parameters, and even nonpathogenic immune challenges can induce relevant physiologic changes in the host (Norris and Evans, 2000). Most of our knowledge about the function and dynamics of immune responses comes from laboratory studies in controlled environments or from studies on inbred individuals of a small number of model species. In contrast, natural populations exhibit wide genetic and environmental diversity, and epizootic diseases often involve vectors and hosts of a variety of species that interact in a complex way (Pedersen and Babayan, 2011).

Adaptive immunity is the most efficient line of defense in vertebrates, as it targets specific antigens, relying on both humoral and cell-mediated immunity to ensure a faster response to subsequent challenges. The humoral response is mediated by lymphocytes T and B that recognize specific antigens, and secrete antibodies to target them for destruction. Likewise, cell-mediated immunity relies on a variety of immune cells that are responsible for antigen recognition and memory, production of antibodies, and the elimination of the specific antigen, among other functions. However, little is known about variability in the early dynamics of those responses and their long-term persistence, which limits their suitability for evolutionary and ecologic studies in the wild.

Immunologic research on wild avian systems has been mostly restricted to nonpasserine species, and the use of antigens such as sheep red blood cells (SRBC) or keyhole limpet hemocyanin (KLH), to which birds have never been naturally exposed. However, the study of adaptive responses to specific wild pathogens can yield relevant information on epidemiologic and evolutionary issues

concerning host–pathogen dynamics (Demas et al., 2011).

Newcastle disease virus (NDV) is a globally distributed avian paramyxovirus that causes highly contagious disease and represents a severe problem for the poultry industry (Alexander, 2009). Few studies have been performed on the specific immune response to NDV, especially in wild populations and nonmodel species (Seal et al., 2000). Vaccination is a common technique widely applied to prevent and alleviate disease and as an immunologic tool to study eco-epidemiology in wild animal populations (e.g., Staszewski and Boulinier, 2004). The development of new in vivo immunologic methods can improve our understanding of host–pathogen dynamics in the wild, potential pathogen reservoirs, and epizootics that may be crucial for endangered species' survival, and the study of passive immunity (e.g., Garnier et al., 2011).

We assessed the immune response of wild House Sparrows (*Passer domesticus*) to immunization with NDV inactivated vaccine. In particular, we aimed to determine the dosage eliciting a significant immune response and the temporal pattern of cell-mediated and humoral responses in adult House Sparrows. House Sparrows are commonly exposed to a variety of pathogens in the wild and are sometimes considered a source of epizootic threats because of the species' close association with humans and livestock (Leighton and Heckert, 2007).

We captured 81 adult House Sparrows in December 2009 in the vicinity of Cañada de los Pájaros bird reserve in Puebla del Río, Sevilla, Spain (37°14′N, 6°07′W). Birds were housed in outdoor cages (3×3×2 m) with ad libitum food and water. On day 2, blood samples (0.2 mL) were taken and individuals were randomly assigned to five treatments: 1–3) inoculated subcutaneously with different doses of a commercial inactivated Newcastle disease virus vaccine HIPRAVIAR® BPL2 $10^{9.5}$ EID_{50}/0.5 mL (0.2 mL, 0.1 mL, or 0.05 mL), 4) inoculated with the same oil adjuvant (0.1 mL) as the commercial vaccine, or 5) inoculated with sterile phosphate buffered saline (0.1 mL). Sampling was repeated at 1, 4, and 6 wk after experimental treatment. In all sampling occasions except in week 4, a blood drop (0.01 mL) was kept in a heparinized tube for determination of leukocyte profile by flow cytometry (see below). Treatment doses were established according to the manufacturer's recommendations and dosages used in previous studies (Saino et al., 2002).

We used hemagglutination inhibition (HI) to assess NDV antibody levels in sera. The HI test is a classic indirect diagnostic assay that is widely used and serves as a gold standard for detecting NDV antibodies in birds (Alexander, 2009). The concentration of NDV antibodies was determined by adding 4 hemagglutinin units of HIPRAVIAR®-CLON (E.Newcastle, clon CL/79) antigen to test sera sequentially diluted from 1:2 to 1:640. VLDIA053 (HAR-NDL, NDV strain La Sota) and VLDIA030 (SPF-CH-Chicken negative) were used as positive and negative controls, respectively. Eleven individuals with initial HI titers exceeding 1:16 were removed from the analyses, as presumably these had been previously exposed to NDV in the wild. Leukocyte profiles were determined following Uchiyama et al. (2005) to estimate relative lymphocyte, granulocyte, and monocyte counts with the use of a flow cytometer (Guava Easy Cyte Plus, Guava Technologies, Hayward, California, USA). Variation in NDV antibody concentration (expressed as the inverse of the dilution factor) was analyzed as the dependent variable in a generalized linear mixed model with normally distributed error and identity link. Comparisons among factor levels were done with post hoc *t*-tests on least-squares means (Fig. 1). Because adjuvant and control treatments did not differ in any of the sessions (data not shown), data were merged into a single

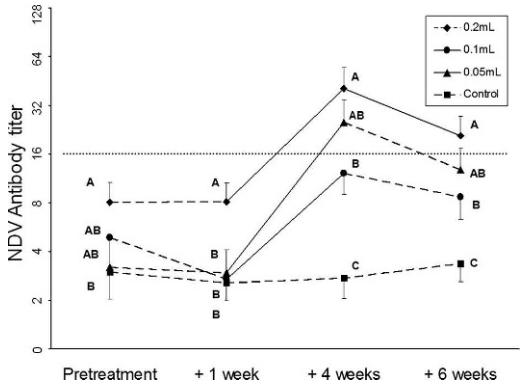

FIGURE 1. House Sparrow (*Passer domesticus*) response to experimental challenge with different doses of inactivated Newcastle disease virus (NDV) vaccine. Least-squares mean NDV antibody titers among experimental treatments on vaccination day (pretreatment), and 1, 4, and 6 wk postchallenge. Values having the same letter within a given experimental session are not significantly different ($P>0.05$). Continuous lines within treatments indicate a significant change between sampling sessions ($P<0.05$). The horizontal dotted line indicates the titer (16) above which samples were considered positive.

control group to improve statistical power. Treatment and session were included as main effects, with cage as a random factor and individual as repeated subject as implemented in proc GLIMMIX SAS 9.2. Additionally, the analyses were repeated with the leukocyte counts as covariates in the previous models, and later as dependent variables with treatment and session as main effects and cage as random factor. Degrees of freedom for fixed effects were estimated by means of the between–within approximation (SAS Institute Inc., 2009). Differences in sample sizes among treatments and sessions are due to a few individuals escaping, and insufficient sera for some laboratory analyses.

Sex ($F_{1,61}=0.06$; $P=0.81$), the interaction between sex and treatment ($F_{3,58}=0.72$; $P=0.54$), body mass ($F_{1,119}=0.04$; $P=0.84$), tarsus ($F_{1,61}=2.11$; $P=0.15$), and wing length ($F_{1,61}=1.00$; $P=0.32$) were initially included as covariates and later removed from the models, as there was no significant effect on the dependent variable.

TABLE 1. House Sparrow (*Passer domesticus*) response to an experimental challenge with different doses of inactivated Newcastle disease virus (NDV) vaccine. Leukocyte profiles as measured by flow cytometry: percentage (±SE) of lymphocytes (Lym), granulocytes (Gra), and monocytes (Mon) among treatments with their corresponding standard error, at the beginning of the experiment, after week 1 and week 6 postchallenge.

Treatment	Pretreatment	Week 1	Week 6
Control			
Lym	0.26±0.03	0.21±0.03	0.16±0.03
Gra	0.70±0.03	0.74±0.03	0.81±0.03
Mon	0.04±0.01	0.05±0.01	0.02±0.01
0.05 mL			
Lym	0.26±0.03	0.21±0.03	0.20±0.03
Gra	0.70±0.04	0.76±0.04	0.78±0.04
Mon	0.04±0.01	0.04±0.01	0.02±0.01
0.1 mL			
Lym	0.27±0.04	0.24±0.03	0.24±0.03
Gra	0.67±0.04	0.73±0.04	0.73±0.04
Mon	0.05±0.01	0.05±0.01	0.03±0.01
0.2 mL			
Lym	0.27±0.03	0.26±0.03	0.18±0.03
Gra	0.70±0.04	0.69±0.03	0.79±0.03
Mon	0.03±0.01	0.04±0.01	0.04±0.01

Concentration of NDV antibodies in response to vaccination varied by treatment ($F_{3,62}=12.55$; $P<0.0001$), and by experimental session ($F_{3,120}=23.55$; $P<0.0001$; Fig. 1). Individuals did not differ in their NDV antibody concentration among treatments at the beginning of the experiment ($F_{3,120}=1.76$; $P=0.16$). However, as indicated by the significant interaction between treatment and session ($F_{9,120}=2.91$; $P=0.004$) differences among treatments appeared after 1 wk ($F_{3,120}=4.13$; $P=0.008$), reached a maximum at week 4 ($F_{3,120}=16.27$; $P<0.001$), and remained unchanged until week 6 ($F_{3,120}=8.21$; $P<0.001$; see Fig. 1 for post hoc tests).

Leukocyte profile changed throughout the experiment as the proportion of lymphocytes ($F_{2,103}=4.84$; $P=0.010$) and monocytes ($F_{2,103}=7.03$; $P=0.001$) declined over time, but the proportion of granulocytes increased ($F_{2,103}=6.53$; $P=0.002$), independently of treatment

(all $P>0.5$, see Table 1). Leukocyte profiles were not related to antibody concentration, either when included singly or together as covariates in the previously described models (all $P>0.5$).

As found in earlier studies on poultry, the inactivated NDV vaccine elicited a significant humoral response in wild sparrows, but did not affect cell-mediated immunity as measured by leukocyte profiles (Popovic et al., 2010). Different treatments did not affect the birds' serology when sampled at week 1 postchallenge, but a serologic response was detected and peaked after week 4, remaining at this level until the end of the experiment. The highest vaccine dose (0.2 mL) induced a positive response in week 1 after treatment, with titers over 1/16; this response remained significantly different from controls from week 4 until week 6, as found by Lloyd and Wernery (2008) in hybrid falcons. Both lower doses of vaccine (0.05 and 0.1 mL) followed the same pattern as described for the highest dose, but the effects were not detectable at week 1 after vaccination and titers remained close to 1/16, which is normally considered as the positive threshold for the diagnosis of exposure to NDV (Alexander, 2009).

We detected no cell-mediated response to experimental vaccination. This agrees with studies questioning the relevance of cell-mediated immunity in the adaptive response to NDV (Reynolds and Maraqa, 2000). All leukocyte profiles (lymphocytes, granulocytes, and monocytes) remained unaltered among treatments. However, leukocyte profiles from all birds changed as the experiment proceeded, most likely as a result of a response to captivity. The ratio of heterophils (i.e., granulocytes) to lymphocytes is related to physiologic stress (Müller et al., 2011; Johnstone et al., 2012). Inactivated NDV vaccine elicits a humoral response in wild House Sparrows with doses above 0.05 mL per individual, and this response was traced from week 4 to week 6 postchallenge. Our results confirm the validity of these in vivo immunologic methods, which can be used in evolutionary, veterinary, and ecoepidemiologic studies on wild fauna.

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