

Evidence of an interannual effect of maternal immunization on the immune response of juveniles in a long-lived colonial bird

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Summary

1. Little is known about the maternal transfer of antibodies in natural host–parasite systems despite its possible evolutionary and ecological implications. In domestic animals, the maternal transfer of antibodies can enhance offspring survival via a temporary protection against parasites, but it can also interfere with the juvenile immune response to antigens.
2. We tested the functional role of maternal antibodies in a natural population of a long-lived colonial seabird, the kittiwake (*Rissa tridactyla*), using a vaccine (Newcastle disease virus vaccine) to mimic parasite exposure combined with a cross-fostering design.
3. We first investigated the role of prior maternal exposure on the interannual transmission of Ab to juveniles. We then tested the effect of these antibodies on the juvenile immune response to the same antigen.
4. The results show that specific maternal antibodies were transferred to chicks 1 year after maternal exposure and that these antibodies were functional, i.e. they affected juvenile immunity. These results suggest that the role of maternal antibodies may depend on the timing and pattern of offspring exposure to parasites, along with the patterns of maternal exposure and the dynamics of her immune response.
5. Overall, our approach underlines that although the transgenerational transfer of antibodies in natural populations is likely to have broad implications, the nature of these effects may vary dramatically among host–parasite systems, depending on the physiological mechanisms involved and the ecological context.

Key-words: host–parasite interactions, IgG, maternal effect, *Rissa tridactyla*, vaccination.

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Introduction

Maternal effects occur when the phenotype of the mother, a result of both the individual's genetic architecture and her environment, directly influences the offspring phenotype. When such effects are adaptive, they can have important consequences for the ecological and evolutionary dynamics of species (Mousseau &

Fox 1998). The maternal transfer of antibodies to offspring is perceived commonly as a maternal effect that increases offspring fitness (e.g. Gasparini *et al.* 2001; Buechler *et al.* 2002; Pihlaja, Siitari & Alatalo 2006). This maternal effect has been well examined in domestic birds, where it has been shown to provide chicks with temporary protection against parasites (e.g. Eidson *et al.* 1982; Smith *et al.* 1994; Hassan & Curtiss 1996; Sahin *et al.* 2003; Al Natour *et al.* 2004). Although the level of exposure to parasites has been shown to affect the transmission of specific antibodies to offspring in natural systems (Gasparini *et al.* 2001, 2002; Buechler *et al.* 2002), very little is known about

the importance of this induced maternal response in evolutionary and ecological terms (Grindstaff, Brodie & Ketterson 2003). In particular, does exposure to an antigen during the course of a breeding season affect the transfer of maternal antibodies during the next breeding attempt in iteroparous species; and are maternally transferred antibodies functional in natural populations, or in other words, can they bind to antigens and/or activate antigen clearance mechanisms (Metzger 1990)?

Functional maternal antibodies may not only confer passive protection to young, but may also affect the development of the juvenile's immune response (Lemke & Lange 1999; Mondal & Naqi 2001; Siegrist 2003). Studies on mice have demonstrated that these antibodies can enhance juvenile immunity (Anderson 1995; Lemke, Coutinho & Lange 2004) and some recent studies in wild bird populations suggest similar results (Gasparini *et al.* 2006; Grindstaff *et al.* 2006). Vaccination studies, however, report that they can also interfere with the juvenile immune response via a mechanism described as a 'blocking effect' (Glezen 2003). When juveniles are exposed to an antigen at an early age and over a short window of time, the presence of passively acquired maternal antibodies may block the immune response and, thus, may reduce the efficiency of a vaccine. In laboratory conditions, the type and dose of the antigen and the timing of exposure all affect the blocking activity of maternal antibodies. These effects can have important consequences for the design of vaccines, e.g. for influenza virus (Pertmer *et al.* 2000). If a blocking effect occurs in natural populations, it would suggest that: (1) maternal antibodies are functional in the juvenile (that is, they influence the juvenile immune response) and (2) there may be a trade-off between the initial protection provided by maternal antibodies and the long-term protection provided by the juvenile's immunological memory. This trade-off could take different forms depending on the characteristics of the host-parasite system involved.

The aim of our study was to examine the functional role of maternal antibodies in an ecological context. More specifically, we report the results of an experiment testing whether the transfer of maternal immunity affects offspring immune response in a natural population of a long-lived colonial seabird, the black-legged kittiwake (*Rissa tridactyla*). The kittiwake is an ideal model for this type of study as this species, like many colonial species, shows high interannual breeding site fidelity and is often naturally exposed to nest parasites during successive breeding seasons (Boulinier, McCoy & Sorci 2001). Using a vaccine to mimic a microparasitic infection, we examined how maternal exposure to an antigen one year affects the transfer of maternal antibodies to the young the following year, and what the consequences of this transfer were for the juvenile immune response when the juvenile was exposed to the same antigen at 1 day old. If our vaccination protocol was effective, we first expected that vaccinated females would show strong, and potentially long-lasting, titres

of specific antibodies after vaccination, and that they would transfer these antibodies to their young 1 year after vaccination. Secondly, if maternal antibodies are functional in natural populations and block the corresponding antigen, we expected that vaccinated chicks from vaccinated females would have an impaired humoral immune response against the same antigen compared to vaccinated chicks from non-vaccinated females. Conformity to these predictions would suggest that the role of maternal antibodies depends not only on the timing and pattern of offspring exposure to parasites, but also on the pattern of maternal exposure and the dynamics of her immune response.

Methods

STUDY AREA AND BIOLOGICAL MODEL

Fieldwork was conducted over three breeding seasons (2002, 2003 and 2004) on Hornøya, an island in Northern Norway (70°22' N, 31°10' E), where approximately 21 000 pairs of kittiwakes breed (Anker-Nilssen *et al.* 2000). Kittiwakes usually lay two eggs, but clutch size may vary from one to three eggs (Thomas & Coulson 1988). Typically, only one clutch is laid during a breeding season. However, if the first clutch is lost, a replacement clutch may be produced. Kittiwakes, like many colonial seabirds, are long-lived and show strong interannual breeding site fidelity (Erikstad, Tveraa & Barrett 1995; Danchin, Boulinier & Massot 1998; Boulinier *et al.* 2002). This can result in the repeated exposure of individuals over the course of their lifetime to parasites present in the breeding area, such as nest-dwelling ectoparasites and the microparasites they vector (Boulinier, McCoy & Sorci 2001). Investigator disturbance in this species has no major effect on breeding success and nest attendance (Sandvik & Barrett 2001), and the content of clutches can be exchanged with no impact on chick survival (Storey *et al.* 1992).

The reproductive success of kittiwakes can vary dramatically among years, notably in relation to food availability (Oro & Furness 2002). In 2003 the kittiwake breeding season in Northern Norway was particularly late, and chick survival was low. As this had a large impact on the sample size of our study, we repeated the experiment the following breeding season (in 2004, see below).

EXPERIMENTAL PROTOCOL

During the laying period of 2002 (late April/early May), 65 individuals were caught on their nests. Birds were marked with a metal ring and a combination of five colour rings that allowed us to identify them from a distance. The study plot used for the experiment was surveyed at least every 3 days for the entire breeding season (beginning of May until end of July) in each of the 3 years. This allowed us to track individual birds that had been captured and marked as adults. Birds

were caught after egg-laying because it is only after this time that they can be captured in association with their nest. It is for this reason that the maternal transfer of antibodies was not studied in the same season as the initial capture. At capture, a blood sample of 0.4 mL was taken from the left ulnar vein using a sterile syringe rinsed with heparin. Blood samples were stored in 1.5 mL tubes and kept cool until centrifugation a few hours later. After blood sampling, each bird was injected subcutaneously in the back of the neck with 0.25 mL of an inactivated vaccine against Newcastle disease virus (NDV; Nobivac PMV, Intervet SA, Beaucauze, France). This vaccine dose corresponds to the standard volume recommended for chickens and pigeons (Eidson *et al.* 1982). We chose this vaccine as it had already been used successfully to control antigen exposure in other ecological studies (see Staszewski & Boulinier 2004). We have little evidence for the natural circulation of NDV in the study population; among 68 adult female kittiwakes tested prior to vaccination (including 20 individuals breeding outside the study plot and sampled in 2002), only one was slightly above the positive threshold (43%, the threshold being 40%) and was not included in the study. All the other individuals were well below the threshold [mean antibodies level 28.1% (95% CI 26.7, 29.5)]. In addition, all eggs and 6-day-old chicks from non-vaccinated females had antibody levels below the positive threshold.

As only females were of interest for our study, captured males (18 of the 65 individuals captured in 2002) were excluded from the study. Sex was determined using morphometric and behavioural criteria (Barrett *et al.* 1985). However, as these criteria are not fully discriminating, all individuals were sexed genetically a posteriori (Jodice *et al.* 2000).

In order to investigate the dynamics of the antibody response of females to the NDV vaccine, 29 of the 47 females were recaptured 13 days (± 2 days) post-vaccination. A second blood sample was taken and these individuals were then revaccinated to potentially boost their immune response and to test the effect of this second exposure to the antigen. Nine revaccinated females and four females vaccinated only once were recaptured on their nest in 2003 to determine anti-NDV antibody titres 1 year after exposure. No difference was found between females vaccinated once or twice (see Results); henceforth, we do not differentiate among these individuals when we speak of vaccinated females. We did not perform a sham control (females caught but non-vaccinated) for this part of the experiment for two reasons: (1) we aimed at maximizing the sample size of vaccinated females that would potentially transmit antibodies to their chicks the year after; and (2) we felt that the specific immune response of nestlings exposed to NDV vaccine at 1 day old would not differ depending on whether their mother was captured or not the year before. Moreover, as non-vaccinated mothers, we used mainly females observed breeding the previous year, even if not captured, and we matched nests by laying

dates, which is likely to have led to the use of females with comparable characteristics.

To investigate the amount of antibodies transferred to the egg yolk, we compared antibody levels in the maternal plasma and egg yolk of 12 vaccinated females and eight non-vaccinated females captured in 2003. Because the 2003 breeding season was poor (see above), and in order to have enough clutches from vaccinated females in 2004 to carry out the experiment, we repeated the vaccination protocol on 38 new females in 2003. The interannual transfer of antibodies from mother to offspring and its effect on the chick's immune response was therefore investigated in both 2003 and 2004.

We collected one egg (chosen randomly with regard to laying order as this information was not known) from clutches of both vaccinated ($n = 13$ nests in 2003, $n = 13$ in 2004) and non-vaccinated females ($n = 13$ nests in 2003, $n = 31$ in 2004). Sample sizes were lower in 2003 because of low reproductive success (see above). These eggs were collected to determine the titres of maternally derived anti-NDV antibodies (i.e. amount of maternal antibodies transferred). Otherwise, clutches from vaccinated and non-vaccinated females (respectively MatAb⁺ and MatAb⁻ eggs) were paired according to laying dates and the remaining sibling egg of each female was moved to a foster nest. The foster nest was also matched by laying date and used to avoid any post-hatching effect of female treatment. In order to increase sample sizes in 2004, the content of nine foster nests (18 eggs) was constituted of two MatAb⁻ eggs (the number of eggs from vaccinated females was necessarily constrained by the availability of previously vaccinated individuals).

In order to maintain the reproductive success of females, eggs from the foster nest were moved to the nests of the vaccinated and non-vaccinated females. Close to the expected hatching date (27 days after laying), daily checks of nest contents were performed. Eggs and chicks were marked with colour so that each chick could be followed individually. Chicks from vaccinated and non-vaccinated mothers did not differ significantly in hatching rank [in the foster nest, eggs from vaccinated females hatched 0.30 (95% CI -0.86, 1.46) days earlier than eggs from non-vaccinated females]. At 6 days old, identification based on colour spots was replaced by the use of metal rings. All chicks were vaccinated when 1 day old (subcutaneous injection of 0.25 mL in the back of the neck; this is the dose used by Eidson *et al.* (1982) for 1 day-old poultry chicks) and regular blood sampling started at 6 days old. Chicks were then followed over the next 3 weeks with visits at 10, 15, 20 and 25 days old (± 36 h) (see Supplementary material, Table S1 for sample sizes). At each visit chicks were weighed, their wing length was measured and a blood sample was taken. Due to a restricted number of visits to the study plots, asynchronous hatching and the disappearance of chicks due to predation, the sample sizes at the different ages varied.

Both the plasma obtained from blood samples and isolated egg yolks were kept frozen until immunological assays. Antibody extractions from egg yolks were carried out following the protocol described by Mohammed *et al.* (1986) and adapted according to Gasparini *et al.* (2001). We determined anti-NDV antibody titres using a monoclonal antibody-blocking enzyme-linked immunosorbent assay (Svanovir ELISA Kit, SVANOVA Biotech, Uppsala Science Park, Uppsala, Sweden). According to kit instructions, antibody titres are expressed as percentage inhibition (PI) values. Large PI values indicate high titres of anti-NDV antibodies in the plasma. The kit outlines 40% as the threshold level for a positive result. This threshold was determined using plasma from pathogen-free individuals (Czifra *et al.* 1996). Below this level, reactivity is due to background noise. The within-plate repeatability of tests was high (98.3%, $F_{14,15} = 110.40$), as was that between plates (95.5%, $F_{21,22} = 39.46$) and for different extractions of the same egg yolk (83.3%, $F_{14,15} = 11.00$).

STATISTICAL ANALYSES

Means are presented with 95% CI. To determine whether antigen exposure affected anti-NDV antibody titres in female plasma 13 days and 1 year after exposure, and to compare these titres with those of non-vaccinated birds, we performed appropriate mean comparison tests. Whenever the data deviated from the normal distribution (Shapiro–Wilk test), we bootstrapped the data 1000 times to assess the validity of our conclusion (Efron & Tibshirani 1993). However, in no case did this deviation alter our results and conclusions; for simplicity, we present only results using standard statistical procedures.

To investigate the factors affecting offspring anti-NDV titres during their first weeks of life, we modelled the dynamics of anti-NDV titres in chicks from vaccinated and non-vaccinated females (i.e. respectively, MatAb⁺ and MatAb⁻ chicks) using generalized additive mixed models (GAMM). We used the library *mgcv* in R (R Development Core Team 2005), based on penalized regression splines and generalized cross-validation to select the appropriate smoothing parameters (Wood 2000, 2003; Wood & Augustin 2002). GAMMs combine the utilities of linear mixed models (Pinheiro & Bates 2000) and generalized additive models (Hastie & Tibshirani 1990) so that random factors, fixed factors and nonlinear predictor variables can all be estimated in the same statistical model. We included the vaccinal treatment of the mother (i.e. MatAb⁺ vs. MatAb⁻ chicks) and year as fixed factors, and the age of the chick as a smooth term. We used foster nest and foster nest nested within treatment as random factors and evaluated the parsimony of alternative models in an analysis of variance (ANOVA) setting using second-order Aikake's

information criterion (AICc) (Pinheiro & Bates 2000). The sample sizes of MatAb⁺ and MatAb⁻ chicks were relatively unbalanced between the 2 years (especially for chicks of 20 and 25 days old), but the same overall pattern was observed within each year. It is for this reason that we pooled measures of the 2 years to determine AICc values. We also investigated a potential difference in growth rate between chicks of the two treatment groups. The growth curves are linear up to 20 days old (Coulson & Porter 1985; Benson, Suryan & Piatt 2003), so we used the age of the chick, vaccinal treatment of the mother (i.e. MatAb⁺ vs. MatAb⁻ chicks) and year as fixed factors and the chick as a random effect to explain the weight of the chick. We used a Cox proportional hazard regression and a Gehan–Wilcoxon test using the library 'survival' in R (R Development Core Team 2005) to investigate potential differences in survival rates between chick groups, as this approach does not require a consistent hazard ratio between groups.

Results

In 2003, 89% (41/47) of females vaccinated in 2002 were observed breeding on the study plot. In 2004, we observed the same pattern; 88% (34/38) of the females vaccinated in 2003 returned to breed in the study cliffs.

EFFECT OF ANTIGEN EXPOSURE ON FEMALE ANTIBODY TITRES AFTER 13 DAYS AND 1 YEAR

Female antibody titres were 17.8% (95% CI 6.7, 28.9) higher 13 days after vaccination compared to before vaccination (paired *t*-test: $t = 3.3$, d.f. = 28, $P = 0.002$) (see Fig. 1). One year later (2003), the antibody titres of

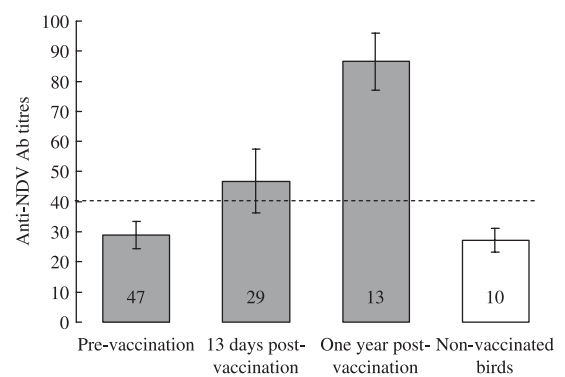


Fig. 1. Anti-NDV antibody titres (percentage inhibition) of females in 2002 and 2003 (mean; SE of the mean; sample size). From left to right, mean antibody titres of females prior to vaccination in 2002, recaptured 13 days post-vaccination, recaptured 1 year post-vaccination (2003), and captured in 2003 but not vaccinated in 2002. The threshold (40%) for a positive response is indicated by the dashed horizontal line. Anti-NDV titres of vaccinated females 1 year post-vaccination were significantly higher than those before vaccination and than antibody titres of non-vaccinated birds ($P < 0.0001$).

a corresponding group of non-vaccinated females were not different from those of vaccinated females before vaccination [1.32% ($-4.67, 7.33$), two-sample t -test: $t = 0.50$, d.f. = 33.77, $P = 0.66$]. They were also below the 40% threshold level for a positive test. As expected, female antibody titres were 66.0% (95% CI 54.5, 77.6) higher 1 year after vaccination compared to before vaccination (paired t -test: $t = 12.59$, d.f. = 12, $P < 0.001$) (Fig. 1).

EFFECT OF MATERNAL ANTIGEN EXPOSURE ON ANTIBODY TITRES IN THE EGG YOLK

The antibody titres of egg yolks from vaccinated females were higher than the titres of egg yolks from non-vaccinated females (t -test: $t = -6.71$, d.f. = 18, $P < 0.0001$). Sample sizes available to compare antibody titres of eggs issued from females that had been vaccinated once or twice were small, but we detected no significant effect of maternal re-vaccination on the levels of antibodies in maternal plasma or egg yolks [mean antibody level of females vaccinated once: 90.3% (95% C.I.: 79.9, 100.0); mean of the females vaccinated twice: 84.9% (95% CI 71.9, 97.9); in maternal plasma t -test: $t = 0.65$, d.f. = 10.3, $P = 0.52$; in eggs $t = 0.70$, d.f. = 4.95, $P = 0.51$]. As predicted, there was a positive relationship between antibody titres of eggs and maternal plasma from vaccinated females (Fig. 2) [$\beta = 1.37$ (95% CI 1.19, 1.55), $n = 12$, $P < 0.0001$]. This relationship was still significant after removing a female that did not respond to vaccination [$\beta = 1.19$ (95% CI 0.88, 1.52), $n = 11$, $P < 0.0001$] (Fig. 2). There was no relationship between maternal plasma and egg yolk titres for the group of non-vaccinated females [$\beta = 0.45$ (95% CI $-0.73, 1.63$), $n = 8$, $P = 0.48$].

EFFECT OF MATERNAL VACCINATION ON THE CHICK'S IMMUNE RESPONSE TO THE SAME ANTIGEN AND GROWTH

The most parsimonious GAMM model involved a random intercept and slope for each foster nest, and a random intercept but constant slope for chicks within each group, MatAb⁺ and MatAb⁻ (results not shown). Anti-NDV antibody titres were lower overall in 2004 than in 2003 [$\beta = -12.26$ (95% CI $-19.98, -4.54$), $P = 0.002$]. However, they were higher among MatAb⁺ than among MatAb⁻ [$\beta = 20.30$ (95% CI 10.44, 30.15), $P < 0.001$] and this effect was consistent between years [test for interaction between year and group: $\beta = 10.98$ (95% CI $-1.89, 23.85$), $P = 0.10$]. As expected, there was a strong interaction between age and treatment group (Fig. 3). Anti-NDV antibody titres of MatAb⁻ chicks increased during the first weeks of life. In contrast, anti-NDV antibody titres of MatAb⁺ chicks were highest just after hatching, and decreased throughout the study period (test for interaction between age and treatment: $\chi^2 = 184.21$, estimated d.f. = 2.73, $P < 0.001$). Approximately 15 days after hatching, the anti-NDV

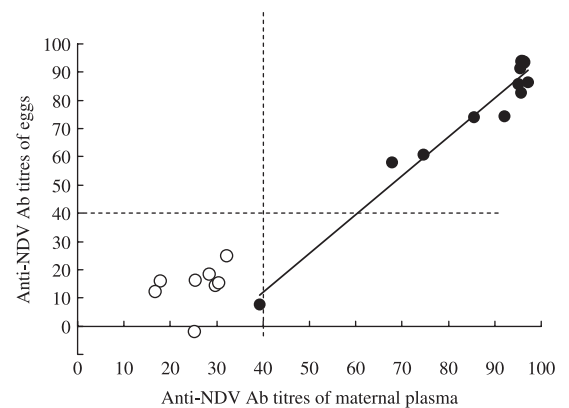


Fig. 2. Relationship between anti-NDV antibody titres (percentage inhibition) of egg yolks and maternal plasma. Filled circles denote titres of vaccinated females ($n = 13$) and open circles denote titres of non-vaccinated females ($n = 8$). There is a positive relationship between the antibody titres of eggs and plasma of vaccinated females. This relationship is still significant after removing the female that did not respond to vaccination (see text). No relationship was found for non-vaccinated females.

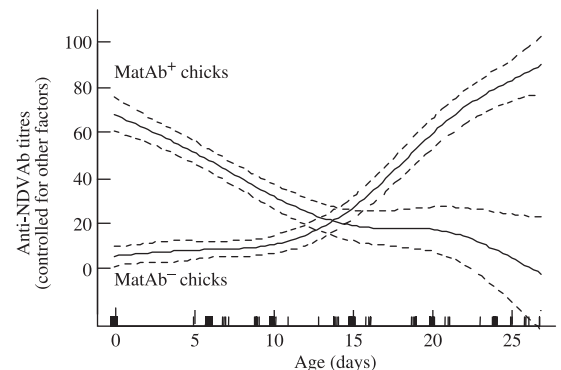


Fig. 3. Mean anti-NDV antibody titres (percentage inhibition) of chicks during the first weeks post-hatching using generalized additive mixed models (GAMM) to control the observed mean anti-NDV titres for factors other than chick group (either MatAb⁺ chicks or MatAb⁻ chicks, i.e. respectively, from vaccinated or non-vaccinated females). Note that the percentage inhibition at age zero is the antibody titre of the sibling egg and that 95% confidence intervals are indicated by dashed lines. The tick-marks on the x-axis indicate the dates at which chicks were sampled (the thickness of the line is a function of the number of individuals sampled). As expected, there was a strong interaction between age and chick group.

antibody titres of MatAb⁺ chicks were significantly lower than that of MatAb⁻ chicks (Fig. 3).

We also tested for potential differences in growth slopes (linear model) between the 2 years and the treatment groups, but found no difference: growth rate in g per day was 14.8 (95% CI 11.2, 18.3) for the MatAb⁺ chicks and $16.0 \pm$ (95% CI 13.3, 18.7) for the MatAb⁻ chicks. The interaction age \times treatment was not significant in either year: in 2003 ($F_{1,15} = 1.89$, $P = 0.18$) and 2004 ($F_{1,62} = 0.06$, $P = 0.80$). In the 2 study years, there

was thus no evidence that offspring grew differently according to their treatment group. We did observe that one group of chicks, the MatAb⁻ chicks in 2004, survived better than the others: using a Cox proportional hazard regression, the interaction year \times maternal treatment showed a significant effect on survival ($P = 0.03$) and a Gehan–Wilcoxon test found that MatAb⁻ chicks in 2004 had a lower mortality compared to MatAb⁺ chicks in 2003 and 2004, but also to MatAb⁻ chicks in 2003 (estimated observed mortality = 6.85; expected = 14.81; $\chi^2 = 15.8$ on 3 d.f., $P = 0.0012$). We had no a priori prediction regarding such survival differences. Given the frequent disappearance of chicks due to predation and the low sample sizes, it is difficult to interpret differences in mortality rates between groups and years.

Discussion

Investigating the ecological and evolutionary importance of the maternal transfer of antibodies from mother to offspring in natural populations requires addressing a series of issues (Grindstaff *et al.* 2003). In particular, in iteroparous animals, does the natural exposure of the mother affect the transfer of antibodies at an interannual scale, and are these antibodies functional (i.e. do they affect immune mechanisms)? Here, we used a vaccination protocol combined with a cross-fostering design to address these issues in a natural population of a long-lived colonial seabird, the kittiwake. Given that parasite exposure often follows a seasonal cycle and that females of many avian species may be present at the nest site for only a few weeks each year (e.g. Loye & Zuk 1991; Randolph *et al.* 2002), our design enabled us to mimic a natural pattern of exposure to nest parasites. The results of our experiment show that (1) exposure to an antigen during the course of a breeding season can lead females to transfer antibodies to offspring during the next breeding season, and (2) maternally transferred antibodies can affect the juvenile phenotype and may interfere with the development of the juvenile immune response.

INTERANNUAL EFFECT OF ANTIGEN EXPOSURE ON THE TRANSFER OF MATERNAL ANTIBODIES

Although a few studies have now investigated the relationship between maternal parasite exposure and the transfer of antibodies to offspring via the egg in natural populations (Gasparini *et al.* 2001, 2002; Buechler *et al.* 2002; Lozano & Ydenberg 2002; Saino *et al.* 2002; Müller *et al.* 2004; Kallio *et al.* 2006), none has considered the effect of maternal exposure to the antigen at an interannual time scale. If maternally derived antibodies are important in natural populations, it should be over long time-periods because there will typically be a delay between the initial maternal exposure and the ability to transfer antibodies to the young. Our

study supports previous results in that females exposed to the parasite (i.e. vaccinated) transmitted specific antibodies to their eggs, whereas non-exposed females did not. However, we show in addition that maternal immunological memory leads to the transmission of these antibodies between years. While some variability in the response of mothers to vaccination was observed, this variation was also seen in antibody titres of their eggs; females that responded more strongly to vaccination transmitted more antibodies to their eggs. In the case of colonial seabirds that are long-lived and relatively faithful to their breeding site, considering individual immunity at an interannual scale is particularly important with regard to life-history decisions, such as where and when to breed (Boulinier, McCoy & Sorci 2001; Møller & Erritzøe 2001). For instance, maternal exposure to parasites before egg-laying may lead to a different response compared to that at hatching or during chick rearing. As maternal antibodies are catabolized within approximately 2 weeks post-hatching in birds (Apanius 1998), potentially adaptive maternal antibodies need to be directed against the parasites which the chicks are exposed to early in order to provide efficient protection (e.g. against nest parasites such as the tick *Ixodes uriae* and its associated bacteria and viruses for nidicolous birds such as kittiwakes; McCoy *et al.* 2002). The immunological memory targeted against nest parasites and acquired during the previous breeding season might therefore be necessary for the transfer of an adequate amount of antibodies to the chicks (Staszewski *et al.* in press). In relation to this, it is interesting to note that antibody dynamics differed between MatAb⁺ and MatAb⁻ chicks over the first 3 weeks of life, a time during which exposure to micro- and macroparasites is likely to be important and vary dramatically between nests (McCoy *et al.* 2002).

FUNCTIONALITY OF MATERNAL ANTIBODIES: INTERFERENCE WITH THE JUVENILE IMMUNE RESPONSE

Several ecological factors are known to affect the transmission of antibodies to offspring under natural conditions (Gasparini *et al.* 2001; Buechler *et al.* 2002; Saino *et al.* 2002; Müller *et al.* 2004). However, it is still unknown whether these antibodies are physiologically active under these conditions (Heeb *et al.* 1998). Our results provide evidence for a blocking effect of maternal antibodies on the chick's immune response. In accordance with our predictions, the transmission of specific maternal antibodies interfered with the response of juveniles to an early exposure to the same antigen. Such a blocking effect has been reported previously in medical (see Glezen 2003 for review) and veterinary studies, particularly in poultry and other domestic mammals (e.g. Chu & Rizk 1975; Giambrone & Closser 1990; Mondal & Naqi 2001). Our study demonstrates that maternally transferred antibodies can also be biologically active under natural conditions.

In the present study, we used a vaccine to mimic microparasite exposure and measured specific antibody titres. However, the production of antibodies after exposure to an antigen is only one type of response among many that are involved in parasite immunity. Immunological responses to macroparasites/ectoparasites can involve components that are not necessarily involved in responses to microparasites, such as the production of IgA, the recruitment of eosinophils and potential trade-off that might exist between these responses (Staszewski & Boulinier 2004). Many bird species are exposed to ectoparasites that can vector microparasites (e.g. ticks, fleas, lice, etc.) and complex interactions are known to exist between the molecular mechanisms used by micro- and macroparasites to fight host immune responses (Wikel 1996). Thus, the impact of maternal antibodies on the development of the juvenile immune system may involve many components and other aspects of juvenile immunity, along with cases of natural parasite exposure remain to be examined.

Here, we investigated a blocking effect of maternal antibodies following exposure to an antigen, but it is important to consider other potential effects. In particular, other studies, mainly in mammals, have suggested that rather than interfering negatively with the juvenile immune response, maternally derived antibodies could have a 'priming' effect; that is, they could enhance the development of the chick's immune response (Gasparini *et al.* 2006; Grindstaff *et al.* 2006; see Anderson 1995 for a review and Bertley *et al.* 2004 for a case study with humans). In our study, chicks were followed for only 25 days and thus a potential 'priming' effect that might occur later in life could not be detected. In this context, it would be particularly interesting to design studies that would enable one to compare the relative fitness and the dynamics of the immune response over an individual's lifetime for individuals exposed to contrasting levels of naturally occurring parasites and having received different levels of maternal antibodies.

More generally, a comparative approach using various naturally coevolved host-parasite systems should also yield insight into the overall importance of maternally mediated immunity. For instance, one may wonder if females of different species have to face an evolutionary trade-off between (1) conferring temporary protection to juveniles and (2) affecting the development of the juvenile's immunological memory. The nature of such a trade-off may be affected directly by the virulence and the timing of exposure to different natural pathogens. A 'blocking effect', although counter-adaptive at first sight, might in fact save the juvenile from using energy to mount an immune response at a time when resources are required for growth and early development (Stearns 1992; Lindström 1999; Zuk & Stoehr 2002). It could also help to prevent the development of immunological tolerance (i.e. misrecognition of pathogens as self) at an early age, thus preserving immunocompetence (Klipper, Sklan & Friedman 2004). Overall, the results of the

present study underline that investigating the evolutionary and ecological implications of the maternal transfer of antibodies cannot be limited to testing the potential temporary and passive protection conferred to the juvenile, but will also need to be considered in light of the relationships between host life histories and the spatio-temporal patterns of parasite exposure.

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Supplementary material

The following supplementary material is available for this article.

Table S1. Sample sizes of chicks vaccinated at 1 day old and sampled at different ages in 2003 and 2004. The analyses considered the exact age of the chicks and not age classes.

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