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Ascaridia galli infection influences the development of both humoral and cell-mediated immunity after Newcastle Disease vaccination in chickens

Janne Pleidrup[a,1], Tina S. Dalgaard[a,1], Liselotte R. Norup[a], Anders Permin[b], Torben W. Schou[b], Kerstin Skovgaard[c], Dorte F. Vadekær[c], Gregers Jungersen[c], Poul Sørensen[d], Helle R. Juul-Madsen[a,*]

[a] *Department of Animal Science, Aarhus University, DK-8830 Tjele, Denmark*
[b] *DHI, Environment and Toxicology, Agern Allé 5, DK-2970 Hørsholm, Denmark*
[c] *DTU Veterinary, National Veterinary Institute, Division of Veterinary Diagnostics and Research, Technical University of Denmark, Bülowsvej 27, DK-1870 Frederiksberg C, Denmark*
[d] *Department of Molecular Biology and Genetics, Aarhus University, DK-8830 Tjele, Denmark*

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ABSTRACT

Potent vaccine efficiency is crucial for disease control in both human and livestock vaccination programmes. Free range chickens and chickens with access to outdoor areas have a high risk of infection with parasites including *Ascaridia galli*, a gastrointestinal nematode with a potential influence on the immunological response to vaccination against other infectious diseases. The purpose of this study was to investigate whether *A. galli* infection influences vaccine-induced immunity to Newcastle Disease (ND) in chickens from an MHC-characterized inbred line. Chickens were experimentally infected with *A. galli* at 4 weeks of age or left as non-parasitized controls. At 10 and 13 weeks of age half of the chickens were ND-vaccinated and at 16 weeks of age, all chickens were challenged with a lentogenic strain of Newcastle disease virus (NDV). *A. galli* infection influenced both humoral and cell-mediated immune responses after ND vaccination. Thus, significantly lower NDV serum titres were found in the *A. galli*-infected group as compared to the non-parasitized group early after vaccination. In addition, the *A. galli*-infected chickens showed significantly lower frequencies of NDV-specific T cells in peripheral blood three weeks after the first ND vaccination as compared to non-parasitized chickens. Finally, *A. galli* significantly increased local mRNA expression of IL-4 and IL-13 and significantly decreased TGF-β4 expression in the jejunum two weeks after infection with *A. galli*. At the time of vaccination (six and nine weeks after *A. galli* infection) the local expression in the jejunum of both IFN-γ and IL-10 was significantly decreased in *A. galli*-infected chickens. Upon challenge with the NDV LaSota strain, viral genomes persisted in the oral cavity for a slightly longer period of time in *A. galli*-infected vaccinees as compared to non-parasitized vaccinees. However, more work is needed in order to determine if vaccine-induced protective immunity is impaired in *A. galli*-infected chickens.

1. Introduction

Worldwide, vaccination is a cost-effective intervention used for control of a wide range of infectious diseases. However, there is increasing evidence that parasite infections confound immune responses to third party antigens and may interfere with vaccine efficacy. Thus, in helminth-infected humans, a reduced humoral and cellular immune response was observed after cholera [1], bacillus Calmette-Guérin [2,3] and tetanus toxoid vaccination [4,5]. In some cases, drug-induced clearance of the helminth infection significantly improved vaccine-specific responses [1,2]. A negative impact of a persistent helminth infection on vaccine efficacy was furthermore described in a porcine model using co-infection with *Mycoplasma hyopneumoniae* and *Ascaris suum* [6]. Pigs were continuously reinfected with *A. suum* eggs and subsequently immunized with killed *M. hyopneumoniae*. Both after immunizations and after challenge with live *M. hyopneumoniae*, the *A. suum*-infected animals developed a significantly lower antibody response against *M. hyopneumoniae* than the non-parasitized animals.

A common parasite species in poultry is *Ascaridia galli*, a gastrointestinal nematode infecting domestic as well as wild birds. As described for helminth infections in mammals, *A. galli* infections in

* Corresponding author. Tel.: +45 87157837.
E-mail address: Helle.JuulMadsen@agrsci.dk (H.R. Juul-Madsen).
[1] Contributed equally.

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chickens are associated with local T cell infiltrations in the intestinal mucosa [7] as well as a Th2 polarized cytokine response [7–9] (& Pleidrup, unpublished observations). A systemic humoral immune response also develops upon *A. galli* infection although serum antibody titres are non-persistent and do not appear to correlate with worm burden or egg excretion rates [7,10,11]. The adult parasite resides in the lumen of the small intestine of the chicken and infection is associated with impaired feed conversion and reduced weight gain [12,13]. The prevalence is up to 100% in chicken flocks with outdoor access [14–17]. Thus, *A. galli* potentially represents a serious problem if the infection hampers disease control through vaccination, e.g. in free range and organic chicken flocks.

Newcastle disease (ND) is a disease of significant economic importance and hence controlled by vaccination in many countries with commercial poultry production [18]. Horning et al. [16] investigated the influence of *A. galli* infection on Newcastle disease virus (NDV) vaccination in naturally parasite-infected indigenous chickens and found that dewormed NDV-vaccinated chickens showed a higher NDV-specific antibody response upon challenge with virulent velogenic NDV. However, neither cellular vaccine responses nor vaccine-induced protective immunity were addressed in the former study. The purpose of this study was therefore to investigate cellular and humoral NDV vaccine responses and compare protective immunity induced in *A. galli*-infected individuals as well as in non-parasitized controls using chickens from an MHC-characterized inbred line in a controlled experimental challenge infection.

2. Materials and methods

2.1. Animals

Chickens from line 133 kept at Aarhus University were used. Line 133 is of White Leghorn origin containing birds with the MHC haplotype B13. The experiment was initiated when the chickens were four weeks of age in order to avoid influence from maternal antibodies. Mixed gender was used. Water and commercial chicken feed were supplied *ad libitum*. The lighting period was 12 h daily, and the chickens were subjected to a temperature of 21 °C.

All chickens used in the experiment were produced from MHC-characterized parents. The MHC haplotypes of the offspring were confirmed by genotyping the LEI0258 microsatellite locus [19] by PCR-based fragment analysis. In brief, red blood cells from peripheral blood were used as template for PCR using the Phusion Blood Direct PCR kit (Finnzymes, Espoo, Finland) according to the manufacturer's instructions. Amplification by PCR and gel documentation were performed as earlier described [20].

2.2. Experimental outline

Chickens were wing-banded at hatch and divided into four different experimental groups; (1) no *A. galli* infection, no ND vaccination (A−N−), (2) no *A. galli* infection, ND vaccination (A−N+), (3) *A. galli* infection, no ND vaccination (A+N−) and (4) *A. galli* infection and ND vaccination (A+N+). Eight animals from each group were used for blood sampling and oral swabs throughout the study. Seven animals from each group were used for spleen and jejunum sampling at each time-point.

The experimental *A. galli* infection (1750 infective *A. galli* eggs/animal) was performed when the animals were four weeks of age (Day 0 PI). At 10 and 13 weeks of age, the chickens were ND-vaccinated using Nobilis ND C2 (live attenuated strain Ulster C2, Intervet). Vaccination days were designated day 0 post-vaccination 1 and 2 (PV1 and PV2), respectively. The Ulster C2 strain is an asymptomatic enteric strain (genotype I) and was given nasally, $>10^{7.5}$ EID_{50}/dose. During the first part of the experiment, vaccinated and non-vaccinated controls were kept in separate units of the facility and within each unit, *A. galli*-infected chickens and non-parasitized controls were placed in separate rooms. At 16 weeks of age (12 weeks PI) chickens were transferred to four different negative pressure isolators and exposed to NDV challenge infection (CH), i.e. the chickens were infected by nasal inoculation of 200 μl LaSota virus (titer $10^{9.5}$ ELD_{50}/ml) diluted 1:100 in PBS. The virus was cultivated by passage in embryonated specified pathogen-free (SPF) hen eggs according to the 92/66/EEC council directive (http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=CONSLEG:1992L0066:20080903:EN:PDF). Titration of the virus was done by inoculation of a 10-fold dilution series in four embryonated chicken eggs for each step.

2.3. A. galli- and NDV-specific IgG ELISA

Blood samples were taken on day 0 PV1 and continuously every week throughout the experiment and serum was used for detection of *A. galli*- and NDV-specific IgG (IgY) antibodies. The *A. galli*-specific IgG titres were measured as described by Norup et al. [11]. Briefly, 96-well microtitre plates coated with *A. galli* soluble antigen (5 μg/ml) were incubated with serum samples, controls or standards. Horseradish peroxidase-conjugated goat anti-chicken IgG (AAI29P, AbD Serotec, Oxford, UK) was used as detection antibody and coloration was driven by tetramethylbenzidine. The results were recorded as the optical density (OD) at 450 nm with 650 nm as reference. A dilution series of a highly positive serum was used as standard. The NDV-specific IgG ELISA was performed as previously described in Dalgaard et al. (2010), using the Flock Check* NDV ELISA kit (Idexx Laboratories Westbrook, Maine, USA) following the kit manual. The antibody titres were calculated from a sample to positive ratio (S/P) as $\log_{10}$ titre = $(1.09 \times \log_{10}$ S/P$) + 3.36$, the S/P being calculated from negative control mean ($\bar{NC}$) and positive control mean ($\bar{PC}$) as (sample mean − $\bar{NC}$/$\bar{PC}$ − $\bar{NC}$).

2.4. Faecal A. galli egg excretion

Faecal samples were obtained from all *A. galli*-infected chickens at day 0 PV1, at day 0 PV2 and at the day of the NDV challenge (day 0 PCH). The faecal samples were examined for the presence of *A. galli* eggs using a modified McMaster counting technique [21,22] with a detection limit of 20 eggs per gram faeces (EPG).

2.5. Real-time quantitative RT-PCR–NDV genomes in oral swabs

Oral swabs were taken from eight animals from each of the four experimental groups at days 0, 3 and 7 PV1 and PV2, respectively, and at days 0, 2, 4 and 7 post-CH (PCH). Sampled swabs were placed in PBS supplemented with penicillin (2000 U/ml), streptomycin (2 mg/ml), foetal calf serum (FCS, 5%) and kept at −20 °C for later analysis. Swab samples from the same group were pooled before RNA isolation from day 0 PV1, day 0 PV2 and day 0 PCH. From all other days RNA was isolated from individual samples. The MagMAX$_{TM}$ 96 Viral RNA Isolation Kit (Applied Biosystems/Ambion, Foster City, CA, USA) was used to isolate RNA from 45 μl swab or swab pool according to the manufacturer's instructions. RNA was eluted in 40 μl Elution Buffer from which 5 μl was used as template for one-step qRT-PCR with Taqman®NDV Reagents and Controls (Applied Biosystems). The qRT-PCR reaction was performed on a ABI PRISM 7900HT instrument (Applied Biosystems) programmed to cycle 10 min at 48 °C, 10 min at 95 °C, 40× (15 s at 95 °C and 45 s at 60 °C). The NDV-specific primers and probe included in the kit were designed to amplify several NDV strains, including LaSota and Ulster, as described in [23].