Veterinary Parasitology 233 (2017) 43–47

Contents lists available at ScienceDirect

Veterinary Parasitology

journal homepage: www.elsevier.com/locate/vetpar

Research paper

Maternal protection against *Ascaridia galli*?

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ARTICLE INFO

Article history:
Received 16 May 2016
Received in revised form
13 November 2016
Accepted 15 November 2016

Keywords:
Chicken
Egg yolk
IgY
Nematode
Protective immunity

ABSTRACT

Maternally derived antibodies can provide partial protection against certain bacterial and viral infections. We investigated whether chicks descending from nematode-infected hens are more resistant against *Ascaridia galli*, a prevalent gastrointestinal nematode, than chicks from nematode-free mothers. One-day-old chicks (N = 153) from infected (mab+; maternal antibody+) or uninfected control dams (mab-) were experimentally infected with *A. galli* at two different levels (100 or 1000 eggs/chick). The worm burdens of the chicks were determined at 6 weeks post infection. There was a high correlation (r = 0.89) between *A. galli*-specific antibody concentrations in dam plasma and egg yolk. There was no difference between worm burdens of chicks descending from infected or uninfected dams (P = 0.892), indicating no maternally derived protection against *A. galli*. Chicks receiving the higher infection dose had higher worm burdens (P < 0.05). Although there was no difference (P > 0.05) between worm counts of female and male chicks infected with 100 eggs, females chicks infected with 100 eggs harboured longer and heavier female worms. We conclude that there is no protective maternal immunity against *A. galli* infection.

1. Introduction

Many studies have shown that maternal antibodies are the primary means of protection against infection in very young chickens (Jungherr and Terrell, 1948; Kowalczyk et al., 1985; Mockett et al., 1987; Heller et al., 1990; Mondal and Naqi, 2001; Akhter, 2003; Hamal et al., 2006; Gharaibeh et al., 2008). Transferring maternally derived antibodies from an immune individual to a non-immune individual via egg yolk confers passive immunity in the recipient (Brambell, 1970). There are three principal classes of homologous antibodies, IgM, IgG (IgY) and IgA to the corresponding mammalian isotypes, and in birds, IgY is found predominantly in the egg yolks rather than the egg white (Leslie and Clem, 1969; Kincade and Cooper, 1973; Dahan et al., 1983; Sharma, 1997; Härtle et al., 2014).

The number of poultry farms with outdoor access, based on consumer demand (Gauly et al., 2002) and the European regulations for laying hens (Anonymous, 1999), has increased. Thus, the roundworm *Ascaridia galli*, is becoming one of the most abundant gastrointestinal nematodes (Permin et al., 1999; Chadfield et al., 2001; Idi et al., 2004; Pleidrup et al., 2014; Wongrak et al., 2014). *A. galli* has direct adverse effects on chicken performance (Ramadan and Abou Znada, 1991; Daş et al., 2010; Das et al., 2012), and can lead to economic losses (Gauly et al., 2005; Daş et al., 2010; Andersen et al., 2013) and increase the susceptibility of chickens to secondary infections (Dahl et al., 2002; Permin et al., 2006). Disinfection and pharmaceuticals are expensive (Gauly et al., 2001). *A. galli* has been controlled mainly by synthetic anthelmintics (Tarbiat et al., 2016). Financial costs, potential anthelmintic underdosing (Bettridge et al., 2014), future anthelmintic resistance (Johnston et al., 2012) and potential anthelmintic residues in food products (Bovenhuis et al., 2002) have led to the need to develop an inexpensive and environmentally friendly method of protecting offspring against *A. galli*. Parasite collagen-based cuticles with carbohydrate-rich surface-coated bodies (Fetterer and Rhoads, 1993), as well as the ability to change antigenic surfaces by moulting several times throughout the development cycle (Blaxter et al., 1992), play a dominant role in how parasitic worms evade by the host innate immune system (De Veer et al., 2007). There are few studies describing naturally acquired immunity against *A. galli* (Bovenhuis et al., 2002).

Chickens experimentally infected with *A. galli* eggs develop both cellular and humoral immunity by secreting Th2-type cytokines and IgY, respectively (Degen et al., 2005; Marcos-Atxutegi et al., 2009; Schwarz et al., 2011). Maternal IgY antibodies can be transferred to progeny via the egg yolk (Marcos-Atxutegi et al., 2009); ovarian follicles, yolk sac membranes, and oviduct secretions are

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http://dx.doi.org/10.1016/j.vetpar.2016.11.014
0304-4017/

the main transfer paths (Kramer and Cho, 1970; Rose et al., 1974; Dohms et al., 1978; Loeken and Roth, 1983; Sharma, 1997; Grindstaff et al., 2003; West et al., 2004). *A. galli* infection can induce an humoral immune response in the host (Edgar, 1971), but this response may not be protective (Andersen et al., 2013; Norup et al., 2013). As far as we know, no studies have been conducted to identify passive immunity to *A. galli* in chickens. Thus, the aim of this study was to investigate whether maternal antibody positive (mab+) chicks are better able to resist *A. galli* infection than maternal antibody negative (mab−) chicks.

2. Materials and methods

2.1. Experimental design and animals

A. galli-free and *A.galli*-infected chickens (N = 20; 36 wk old) of Lohmann Selected Leghorn (LSL) genotype were obtained from a research farm (University of Göttingen, Germany) and kept in two disinfected (INTERKOKASK, *p*-Chlor-*m*-kresol, 4%) barns as control and infected groups, respectively. Nematode free and flubendazole treated cockerels (LSL, 18 wk) were placed in these barns for two weeks to mate (sex ratio: 1/10). Afterwards, all laid eggs were collected at regular intervals, and the positive fertility results for some of the randomly collected incubated eggs (N = 37) demonstrated successful mating. All collected eggs were numbered (N = 198) and incubated. Dams were necropsied, and individual blood and egg yolk samples were collected. A total of 153 newly hatched chicks (mab+, mab−) were challenged orally by infection (mono-infection) on day 1 with embryonated *A. galli* eggs, using a 5 cm oesophageal cannula (Daş et al., 2010). Half of the chicks were challenged with +100 *A. galli* eggs, and the remainder were infected with a 10-fold higher infection dose (+1000). At necropsy (6 wk post infection), blood was collected from all birds, and they were subjected to post-mortem parasitological examinations by sieving (100 μm mesh aperture size) the whole small intestinal content. Worms visible with the naked eye were recovered, and microscopy was used to assess the presence of larvae not visible with the naked eye. The demographic characteristics of the infrapopulations were then determined.

2.2. Infection material

Fully embryonated uterine eggs that were incubated in 0.1% potassium dichromate ($K_2Cr_2O_7$) at room temperature were used for experimental infection(Rahimian et al., 2016).

2.3. A. galli-specific antibody

Plasma *A. galli*-specific antibodies raised against both soluble and solubilized worm body antigens were determined using antigen coated microtiter wells. Bound antibodies were measured by an enzyme-conjugated secondary antibody against chicken IgY as described by (Hennies et al., 2013). At necropsy blood was collected to obtain plasma. Egg yolks were separated and stored −20 °C until analysed. For analyses, the egg yolk and plasma samples were diluted 1:500 and 1:2500, respectively, with test buffer. Due to the tough texture of the yolk, additional mixing was undertaken via vortexing at room temperature to minimize any differences between the samples. Briefly, 100 μl of plasma or egg yolk and standards (all diluted in the test buffer) was added to the coated wells. Dilution series of highly positive plasma samples were used. After incubation (wrapped and placed on a shaker (500 rpm)) for 2 h at RT, washed plates (wash buffer) were filled with 100 μl of enzyme-conjugated anti-chicken IgY, wrapped again and incubated on the shaker (500 rpm) for 30 min at RT. Afterwards, the plates were washed again, and 100 μl of substrate solution (TMB) was added, and the plates were incubated for an additional 15 min at RT in the dark. Colour development was stopped with a stop solution (100 μl) and the plates were read at an absorbance of 450 nm, with the absorbance at 650 nm serving as the reference wavelength. The standard curve described the relation between the concentrations of the standards and their absorbance value generated for each plate. Antibody binding was expressed relative to a standard chicken serum with high antibody activity (1 Unit per mL per definition).

2.4. Statistical analysis

The experiment was subjected to a factorial design. Plasma transferred antibody concentrations were calculated to investigate the occurrence of transferring antibodies via egg yolk. Data for each dependant variable (i.e., plasma antibody levels, worm burden, number of female and male worms, lengths and weights of worms of both genders) were analysed via factorial ANOVA, as shown in the following statistical model:

$$\begin{aligned} Y = {} & 1\mu + B1 \times \text{mother\_inf} + B2 \times \text{chick\_inf} + B3 \times \text{sex} + B4 \times \text{housing\_in} + (\text{mother\_inf} \times \text{chick\_inf} \times \Upsilon_{\text{mother\_inf} \times \text{chick\_inf}}) \\ & + (\text{mother\_inf} \times \text{sex} \times \Upsilon_{\text{mother\_inf} \times \text{sex}}) + (\text{mother\_inf} \times \text{housing\_in} \times \Upsilon_{\text{mother\_inf} \times \text{housing\_in}}) + (\text{chick\_inf} \times \text{sex} \times \Upsilon_{\text{chick\_inf} \times \text{sex}}) \\ & + (\text{chick\_inf} \times \text{housing\_in} \times \Upsilon_{\text{chick\_inf} \times \text{housing\_in}}) + (\text{sex} \times \text{housing\_in} \times \Upsilon_{\text{sex} \times \text{housing\_in}}) \\ & + (\text{chick\_inf} \times \text{housing\_in} \times \text{mother\_inf} \times \Upsilon_{\text{chick\_inf} \times \text{housing\_in} \times \text{mother\_inf}}) + (\text{mother\_inf} \times \text{infection} \times \text{sex} \times \Upsilon_{\text{mother\_inf} \times \text{infection} \times \text{sex}}) \\ & + (\text{chick\_inf} \times \text{housing\_in} \times \text{mother\_inf} \times \text{sex} \times \Upsilon_{\text{chick\_inf} \times \text{housing\_in} \times \text{mother\_inf} \times \text{sex}}) + \varepsilon \end{aligned}$$

Where Y is the observation, 1 is the vector of size n × 1 with each entry equal to one, μ is the fixed effect, and ε is the residual random error. Tukey-Kramer adjusted post-hoc comparisons ($p < 0.05$) were employed to partition the effects of the factors when needed. Shapiro normality tests were used to test for normality of variation among the residuals, and the plots were visually inspected. Worm burden, number of female and male worms were analysed in a generalized linear model specifying a negative binomial distribution. Goodness of fit ($p > 0.05$) was considered for accuracy of the model. Logarithm transformation was used to normalize the data regarding antibody levels. The square root of male worm weight was used, and outliers in male worm length/weight were removed. Worm burden data that were analysed included outliers. All analyses were performed using the R package (Colditz, 2008).

3. Results

There was a strong correlation ($r = 0.89$, $P < 0.001$) between *A. galli*-specific antibody concentration in plasma and egg yolks of infected dams (Fig. 1). The first, median and third quartiles for antibody titres for dam plasma and egg yolk were 23.55, 48.10, 98.55 and 20.7, 31.5, 53.6, respectively. There was no difference between worm burdens of chicks descending from infected or uninfected dams ($P = 0.892$). Chicks challenged with +1000 *A. galli* eggs harboured higher worms ($P \leq 0.001$) than chicks challenged with +100 *A.galli* eggs (Table 1). The higher worm burdens