Genetic Resistance To Natural Helminth Infections In Two Chicken Layer Lines

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Introduction

Traditional control of gastrointestinal (GI) nematode infection has heavily depended on the use of anthelmintics (Woolaston and Baker, 1996). The possible disadvantages are the development of drug resistance (Jackson and Miller, 2006), the relatively high costs, the possible negative ecological impact and chemical residues (Sangster, 1999). Therefore alternative control strategies need to be adopted (Heckendorn et al., 2009). Propably the use of genetic differences in resistance can be one. Gauly et al. (2002) estimated heritabilities for mean log Faecal egg counts (FEC) in white (Lohmann LSL) and brown (Lohmann Brown) laying hens artificially infected with embryonated *A. galli* eggs at an age of 20 weeks between 0.13 and 0.19 for white and 0.0 and 0.10 for brown layers. The same authors recently estimated heritabilities for logarithm (ln) worm burden in two chicken layer lines when artificially infected with 100 embryonated *H. gallinarum* eggs at an age of 8 weeks. Estimates were between 0.41 (SE ± 0.09) in White Leghorn (WL) and 0.31 (SE ± 0.13) in New Hampshire (NH), respectively (Gauly et al., 2008). The experimental technique eliminates possible between-hens differences in larval intake during natural infection. Therefore the presented estimates of heritabilities could underestimate the values of animals exposed to natural infection with parasites (Barger and Dash, 1987). However, the role of genetics can also be expected in the case of natural mixed helminth infections, but so far no heritability estimations for parameters of genetic resistance in chickens were done under such circumstances. The aim of this study was to estimate the genetic resistance of two genetically distinct chicken lines following a natural infection with various helminths based on worm burden.

Material and methods

Animals and management. One day old female chicks with defined origin were used in the study. The chicks originated from two different commercial lines (Lohmann Selected Leghorn (LSL), n = 339); Lohmann Brown (LB), n = 254) maintained from Lohmann Tierzucht GmbH, Cuxhaven, Germany. Within each line, offspring were produced by mating each of 20 sires, representing different sire families, to 10 dams each. From both lines an average of 17 daughters per sire were used in the study. In maximum two descended from one hen. All wing tag marked animals were raised in one floor system. At an age of 19 weeks the animals were brought to a commercial layer farm and kept in a floor husbandry

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system. All hens (n = 930) were kept together in one herd (6 animals per m^2), The animals were helminth-free at that time as confirmed by faecal examinations. A commercial diet and water were provided *ad libitum*.

Mortality rate, clinical examinations and performance. Mortality rate (%) was recorded during the whole laying period. Number of eggs (white and brown) belonging to the different commercial weight categories were recorded on a daily basis. Furthermore, beginning with an age of 20 weeks, 20 animals per line were randomly selected every second month to record their body weights.

Faecal egg counts (FEC). After the 20 animals per line were weighed, individual faecal samples were collected from them to quantify FEC using a modified McMaster technique.

Worm burden. 246 LSL and 197 LB were harvested at the end of the laying period (12 months) following the World Association for the Advancement of Veterinary Parasitology (W.A.A.V.P.) guidelines for evaluating the effectiveness of anthelmintics in chickens and turkey (Yazwinski et al., 2003).

Parasite processing and identification. All visible parasites were collected first, and then the content of the gastrointestinal tract and the scraped mucosa was examined under 20x dissecting microscope. Parasites were counted and stored until differentiation in tap water. Nematodes were identified depending on morphological parameters using the helminthological keys according to Soulsby (1982) and Norton and Ruff (2003).

Statistical Analyses. Statistical analyses were performed applying mixed model methodology as available in the statistical package SAS Version 9.1. Worm burden data were log transformed [log(worm burden+10)]. Data related to worm burden were analyzed with a general linear model including the fixed effect of breed and the random effect of the sire within breed. For worm count analysis the following model was used: $Y_{ijk} = \mu + br_i + sire(br)_{ij} + e_{ijk}$ (Y_{ijk}= observation for the trait, μ = overall mean effect, br_i = effect of breed, $sire(br)_{ij}$ = random sire effect nested within breed, e_{ijk} = random residual effect). Data related to FEC were log transformed [log(FEC+25)] and analyzed with a general linear model including the effect of genotype. The analyses were done for each sampling time separately. The same model was applied for the body weight data that were taken at different sampling dates. Heritabilities stratified by breed were estimated within an animal model using REML-methodology and the program VCE4, version 4.2.5 (Neumaier and Groeneveld, 1998). The following animal model was used: $Y_{ijk} = \mu + a_i + e_{ijk}$ (Y_{ijk}= observation for the trait, μ = overall mean effect, a_i = random additiv genetic animal effect, e_{ijk} = random residual effect). Heritabilities and genetic correlations for the whole dataset, i.e. including animals of both breeds, were estimated applying the following animal model: $Y_{ijk} = \mu + a_i + br_i + e_{ijk}$ (Y_{ijk}= observation for the traits, μ = overall mean effect, a_i = random additiv genetic animal effect, br_i = fixed effect of breed; 1 = LB, 2 = LSL, e_{ijk} = random residual effect).

Results and discussion

Mortality rates and performance. Significant differences ($P < 0.01$) were observed in the mortality rates between LSL and LB animals (12.9 vs. 5.7 %), whereas laying performance was not significantly different between the lines. The values were in accordance with other reports and breeder information as the percentage of eggs belonging to the different commercial weight was.

Faecal egg counts (FEC). FEC increased from 0 (sampling at the time of housing) to an average of 402 in LB and 851 in LSL at the time of third sampling (month 5 to 6), respectively. Afterwards FEC decreased in both lines. 3rd and 4rd samples were significantly ($P < 0.05$) higher in LSL when compared with LB hens. FEC was probably mainly caused by adult female *A. galli* worms. This worm shows a higher fecundity when compared with *Capillaria* spp. (Tompkins and Hudson; 1998). *H. gallinarum* eggs are mainly dropped separately with caecal faeces and therefore not counted in normal droppings (Püllen et al., 2008). This may explain the higher faecal egg counts in LSL which had a higher *A. galli* burden but lower total worm load. This clearly proves the limits of using FEC as an indicator of worm burden under conditions of mixed infections in chickens. The decrease of FEC after six months was probably caused by the development of a host immunity.

Worm burden and species. 99.2 % (n = 244) of the LSL and 98.5 % (n = 194) of the examined LB hens were helminth positive. The following species were found: *Ascaridia galli, Heterakis gallinarum, Capillaria spp.*, *Acuaria hamulosa, Raillietina cesticillus, Hymenolepis cantaniana, Hymenolepis carioca* and *Choanotaenia infundibulum.* Number of adult *A. galli* worms tended ($P = 0.08$) to be higher in LSL hens than in LB hens (7.3 vs. 9.9). However, LB hens harboured significantly ($P < 0.05$) higher numbers of adult *H. gallinarum* (162 vs. 76.5), *Capillaria spp.* (20.7 vs. 7.1) and tapeworms (2.3 vs. 0.8).. Therefore the total mean worm burden was significantly ($P < 0.05$) higher in LB than LSL (192.3 vs. 94.3). The helminth prevalence (Abdelqader et al., 2008; Maurer et al., 2009) and the range of the species found are in accordance with earlier studies (Poulsen et al., 2000). The different development of protective immunity in the hens (Marcos-Atxutegi et al., 2009) may explain the differences between the hens, lines and sires. However, LB animals showed significantly more *Capillaria spp.* when compared with LSL. Even if most of these species also occurs in the small intestine almost nothing is known about immune mechanism in birds. Maybe the degree of immunity varied in the different parts of the intestine. This seems also to be the case for *H. gallinarum* and tapeworms and may explain why LB animals showed significantly more adult worms of this species.

Phenotypic correlations and genetic parameters. Number of *H. gallinarum* was highly correlated (r = 0.94 - 0.96) with total worm burden in both lines. This may indicate that somehow resistance is acting the same way within the lines even if helminths are located in very different parts of the intestine or immunity itself is in a better position if a single worm species is decreasing in numbers. This will be benefical for selection. Similar results were found in sheep (Kemper et al., 2009).

The estimated heritabilities for total worm burden were 0.23 (SE ± 0.12) in the LSL, 0.75 (SE ± 0.21) in the LB hens and 0.66 (SE ± 0.13) over both genotypes, respectively. Heritability estimated for the worm number of the different species ranged between 0.01 and 0.69 (table 1). Estimated heritabilties for body weights at slaughtering were 0.65 (± 0.14) for LB and 0.40 (± 0.12) for LSL, respectively. This values are mainly in agreement with earlier studies (Gauly et al., 2002, 2008) beside the high value in LB hens for *H. gallinarum*. The relatively high standard errors are maybe caused by the limited number of animals used in the study. However, even if the values are over-estimated in the case of LB or under-estimated for LSL this clearly proves the existence of genetic resistance or variation in chickens. Furthermore, the values estimated over both genotypes agree with heritabilities estimated for nematode resistance in sheep, where breeders have started to integrate this parameter into breeding programs (Vanimisetti et al., 2004).

Table 1: Heritabilities (± SE) estimates for the no. of worms in LB and LSL hens

Helminth species	LB	LSL	LSL and LB
Ascaridia galli	0.11 (± 0.07)	0.13 (± 0.06)	0.10 (± 0.06)
Heterakis gallinarum	0.69 (± 0.20)	0.30 (± 0.11)	0.68 (± 0.07)
Capillaria spp.	0.18 (± 0.07)	0.01 (± 0.02)	0.08 (± 0.04)
Tapeworms (all)	0.28 (± 0.12)	0.05 (± 0.05)	0.08 (± 0.05)
Total	**0.75** (± 0.21)	**0.23** (± 0.12)	**0.66** (± 0.13)

Conclusion

In conclusion, selection of chickens for nematode resistance using worm count should be sustainable in the medium to long-term. Estimates found in this study suggest that it is possible to select for helminth resistance in both chicken lines based on worm count. Our results support the hypothesis that nematode resistance is determined by many genes each with relatively small effect. However, selection in chickens can not be done based only on FEC as the most important species are maybe not represented.

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