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QTL detection for coccidiosis resistance using SNPs N. Bacciu^{1,2}, O. Demeure^{1,2}, B. Bed'Hom³, D. Zelenika⁴, F. Pitel⁵, P. Le

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Introduction

Coccidiosis is the most important parasitic disease affecting poultry production. There are several species of chicken coccidia, each having a particular host location and characterized by a specific pathogenic effect such as characteristic gross lesions. Eimeria tenella is one of the most frequent ones, developing in the caecum, affecting feed conversion, causing depression of body weight gain, lesions and in the most severe cases, mortality. The use of genetic resistance has the potential to limit the negative and costly effects of the disease and hence would be relevant to implement. Genetic variability for resistance to coccidiosis in chicken has been extensively proven to exist (Johnson et al. (1986)) and using genetic resistance to coccidiosis would be an attractive control measure. The objective of this present study was to identify QTL for different resistance traits to *E. tenella*. The chosen strategy was to produce, challenge and genotype a F2 cross from two chicken lines, a Fayoumi line and a Leghorn line, identified previously as resistant and susceptible for E. tenella, respectively. The analysis of the F2 and results of the genome scan are presented here (Pinard et al. (2009)).

Material and methods

Fayoumi × White Leghorn cross. An F1 cross was produced using 3 cocks and 7 hens from the Fayoumi line and 3 cocks and 6 hens from the Leghorn line. From this F1 generation, 104 animals (males and females) were tested for E. tenella. Non-challenged full brothers and sisters were used to produce the F2 cross: 6 F1 cocks, each mated to 5 F1 hens, being sisters or half-sisters, produced 860 F2 belonging to both genders (Pinard et al. (2009)). The following resistance criteria were measured: Mortality was recorded until 8 d after inoculation. Body weight gain (WG) was measured as WG = $100 \times$ (Body Weight (8 d post inoculation) - Body Weight (2 d before inoculation))/Body Weight (2 d before inoculation). From blood sampling at 4 d post inoculation, plasma coloration (PC), as a measure of blood carotenoid level, was analyzed as PC = log10(Optical Density at 480 nm) and hematocrit level (HEMA %) was recorded. Rectal body temperature (T°) was measured 4 d post inoculation. At slaughter, cecal lesion scores (LES) were assessed from 0 (no lesion) to 4 (Pinard et al. (2009)).

Genotyping. The F1 sires were genotyped for a set of 9216 SNPs. For the F2 genotyping, a

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panel made up of 157 microsatellite and 1536 SNPs markers covering all the sequenced genome was choosed based on the markers informativity and location using MarkerSet (Demeure and Lecerf (2008)).

Statistical analysis on phenotypes. One of the goal of this study was to obtain some indexes to best resume the aforementioned resistence cirteria to coccidiosis in chicken with a latent variables model. A factor analysis (Krzanowski (2000)) by using *Proc Factor* by SAS was conducted and it allowed to identify one first order factor. The pattern of the new variable shows a very high and positive correlation with WG, HEMA and PC; on the other hand a negative correlation with LES points out the good description of the traits by using the new variable F1. Moreover about 74 % of the original variance can be explained by the only extracted factor. In addition, data were corrected for sex and hatch effect.

QTL detection analysis. MendelSoft (De Givry *et al.* (2005)) was used in order to correct data for possible mendelian errors in the genotype information and QTL detection was performed by using QTLMAP software (Elsen *et al.* (1999)). An Interval Mapping method was applied and no assumption either about fixation of the alleles in the founder lines or the number of the alleles segregating at QTL were made. A mixture of half and full sib families was considered as pedigree structure. Significance thresholds were evaluated through empirical calculations obtained from permutations (discrete trait) (Doerge *et al.* (1996)) or simulations under the null hypothesis. Finally, several approaches were used in terms of model to fit and considered traits according to 1) the distribution of the traits, 2) a possible pleiotropy when more than one QTL were co-segregating at the same location. The following approaches were used: a single QTL and Single Trait detection for continuous traits (ST), a single QTL and Single Trait detection for Discrete traits (STD) and a MultiVariate approach (MV) by combining two ore more traits (Gilbert and Le Roy (2003)).

Results and discussion

Results of the QTL analysis are summarized in table 1.

ST and STD detection. Results clearly show one very significant QTL for LES (< 1%) on GGA18 at 76.38 cM and 3 more co-located and very significant QTLs were detected for WG, F1 and LES on GGA16 at 0.25 cM. Several significant QTLs (<5%) were also detected for HEMA on GGA3, GGA10 and GGA15; for LES on GGA5; for T° on GGA7, GGA11 and GGA17; for F1 on GGA14.

MV detection. An extremely significant QTL was detected on GGA16 at 0.25 cM combining WG and LES to perform a multivariate analysis. These results could be view in confirmation of the ST analysis with a more probative support. Withal correlation structure among traits can strongly affect results: the same QTL location was identified when PC was considered in the multivariate analysis; in this case the power still increased but we came up with a lower significance for the detected QTL. MV strategy allowed the detection of a very significant QTL on GGA4 by combining PC and LES information and ST permitted to detect a suggestive QTL for PC roughly in the same location (result not showed). In the light of this

it seems that MV analysis let some increase of statistical power to be achieved. A similar situation is reported for GGA3 when we decided to combine T° and PC information and for GGA14 where LES information was added to perform an MV the analysis. Additionally MV analysis permitted to achieve the detection result when single QTL analysis was not powerful enough: that is the case for GGA1 (suggestive QTL for the WG-HEMA-PC traits combination) and GGA2 and GGA22 (significant QTL for PC-LES and WG-LES respectively).

Chromosome	Trait(s)	Loc (cM)	LRT	Chr wide	Gen wide
2	PC LES	169.28	123.26	*	
3	HEMA	269.21	76.79	*	
3	$T^{\circ} PC$	193.21	123.70	*	
4	PC LES	224.54	134.24	**	
5	LES	40.37	73.24	*	
7	Τ°	105.24	73.07	*	
11	Τ°	26.06	66.59	*	
14	F1	159.17	65.92	*	
14	PC LES	14.17	115.60	*	
15	HEMA	31.49	68.66	*	
16	WG	0.25	64.11	**	
16	F1	0.25	60.61	**	
16	LES	0.25	68.12	**	
16	WG PC LES	0.25	145.58	**	
16	WG LES	0.25	114.86	***	*
17	Τ°	26.40	69.46	*	
18	LES	76.38	78.52	**	*
22	WG LES	210.73	114.34	*	

Table 1. Estimation of QTL for resistance traits to coccidiosis after inoculation with *E. tenella* in an F2 cross.

* 5% ** 1% *** 0.1%

Conclusion

From an F2 cross of resistant and susceptible chicken lines, 10 chromosome-wide significant QTLs on 7 chromosomes (ST analysis) and 7 chromosome-wide significant QTLs on 6 (MV analysis) were detected. Results show that MV analysis permits to achieve an increase of statistical power in spite of the higher number of parameters to be estimated. Use of the latent variable F1 permitted to detect a significant QTL at 159.17 cM on GGA14 whereas no ST or MV analyses were able to. This could suggest a better description of the overall phenomena when both multivariate methods are used. This study is a good starting point for further gene identification and delineation of underlying mechanisms and hopefully opening possibilities for new breeding strategies including improved resistance to coccidiosis in the chicken. The next step will consist in detecting possible interacting QTL through epistasis analysis.

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