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► To cite this version:

Marie-Hélène Pinard-van Der Laan, Bertrand Bed'Hom, Nicolas Bruneau, Olympe Chazara, Badi Besbes. Major Histocompatibility Complex plays a role in survival kinetics of chicken infected by Marek's disease virus. 10. World Congress on Genetics Applied to Livestock Production (WCGALP), Aug 2014, Vancouver, Canada. 2014, Proceedings, 10th World Congress of Genetics Applied to Livestock Production. hal-01193932

HAL Id: hal-01193932

<https://hal.science/hal-01193932>

Submitted on 3 Jun 2020

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Major Histocompatibility Complex Plays a Role in Survival Kinetics of Chicken Infected by Marek's Disease Virus

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ABSTRACT: Chickens from a Brown Leghorn line were challenged with Marek's Disease Virus (MDV). Mortality and all visceral and nerve lesions were daily recorded. Three Major Histocompatibility Complex (MHC) haplotypes were characterized using genetic markers from the *MHC-B* region. MHC had a significant effect on the kinetics of mortality from MDV, one genotype being associated with an earlier death. Survival analysis showed a significant difference between the birds dying with or without visceral lesions, animals without visceral lesion (but with or without nerve lesions) dying less and later. Heritability of survival time was estimated to be 0.41, indicating potential for genetic improvement. Further analysis should refine the economic interest of using MHC as genetic markers to select for improved resistance to MDV and explore further polymorphisms associated with resistance mechanisms using a survival analysis associated with visceral lesion recording.

Keywords: Poultry; Survival; Marek's disease; MHC

Introduction

Marek's Disease Virus (MDV) is a herpes virus causing still important economic losses to poultry industry despite vaccination programs, as seen by recent outbreaks due to virulent strains (e.g. Gong, (2013)). Genetic resistance to MDV is clearly of polygenic nature, although the effect of some MHC alleles is well documented. But the effect of the different alleles and their combination vary between populations. Also, nearly all the studies have been performed in White Leghorn and scarcely on Brown lines where the determinism of MDV resistance as well as the characterization of MHC are barely known.

The objectives of this study were to 1- identify and characterize segregating MHC alleles in a commercial line of Brown Leghorn and 2- estimate the effect of MHC on survival to MDV infectious challenge, and 3- analyse the determinism of MDV resistance by performing a survival analysis, giving insight into underlying mechanisms.

Materials and Methods

Birds and Marek's Disease challenge. Chickens produced from a commercial Brown Leghorn layer line were used. A total of 378 pedigree females originating from families segregating for MHC haplotypes were produced.

Non vaccinated birds were inoculated at one week old with the "HPRS16" MD strain maintained at ANSES (Ploufragan, France) which causes up to 100% mortality within 12 weeks in SPF White Leghorn population equally challenged as reference. Ages at first symptom and at death were daily recorded until 12 weeks post inoculation. All birds, including the survivors, were autopsied. Type ("visceral": lung, liver, spleen, kidney, proventriculus and "nerve": brachial, sciatic, abdominal vague) and severity of the lesions were individually recorded.

Survival analysis. Survival to MDV was analysed per type of lesions (visceral or nerve). To estimate the survival of birds which died with visceral lesions, birds which died without visceral lesion and survivors were considered as censored. The other way around, to estimate the survival of birds which died with nerve lesions, birds which died without nerve lesion and survivors were considered as censored. The same analysis was conducted per organ within type of lesion.

The survival model was also used to estimate the genetic parameters (heritability) of the time of death, including the MHC genotype, father and mother effects. The Survival Kit software Version 5.1 (Ducrocq and Solkner (1998)) was used for the survival analysis. Ages at first symptom, time to death and death were normally distributed. Effects of MHC genotype on these traits were analysed with χ^2 (Survival Kit) and F (SAS).

Major Histocompatibility Complex typing. The genotype at MHC locus has been determined with DNA extracted from blood samples, using markers LEI0258, GAB0001 GAB0002, all located in the B region of chicken MHC. A triplex PCR was performed on the DNA samples, with the forward primer of each marker fluorescently labelled on its 5' end (with HEX for *LEI0258* and FAM for *GAB0001* and *GAB0002*). The sequence and position of primers in galGal4 chicken reference assembly are in Table 3. PCRs were done in a volume of 10 μ L with 100 ng of DNA, 1X of QIAGEN Multiplex PCR Kit and primer concentrations of 0.1 μ M with the following conditions: 94°C for 15 min, then 35 cycles at 94°C for 45 s, 60°C for 1 min 30 s, 72°C for 1 min, with a final extension at 72°C for 15 min. After a 1:20 dilution, the PCR products were loaded on an Applied 3730XL automated sequencer for electrophoresis and the results were analysed using Genemapper v3.7. The allele sizes were compared with the data published in Fulton et al. (2006), Chazara et al. (2011),

Chazara et al. (2013) and results previously obtained in the laboratory on serologically defined samples.

Results and Discussion

Major Histocompatibility Complex analysis and effect on MDV infection. A total of 357 birds could be typed for the three markers, *LEI0258*, *GAB001* and *GAB0002*, displaying a unique combination of three distinct MHC haplotypes called in this study A, B and C, the latest being the most frequent (74%) (Table 1). The three haplotypes were present in the six possible genotypes, the AC, BC and CC being most present (Table 2) and further analysed for their effect on survival from MDV infection. The BC genotype showed to be most susceptible, BC birds showing MDV symptoms and dying earlier than the AC and CC genotypes whereas CC birds were dying the “slowest” (Figure 1; Table 4).

Table 1. The MHC haplotypes (HAPLO): combination of B genes identified.

HAPLO	<i>GAB0001</i>	<i>GAB0002</i>	<i>LEI058</i>	No
A	159	354	357	96
B	187	322	307	87
C	159	322	309	531

GAB0001, *GAB0002*, *LEI058*: in base pairs
No: Number of clones sequenced.

Table 2. The MHC genotype (Geno) distribution (%) in the 357 animals.

Geno	No	%
AA	3	0.84
AB	9	2.52
AC	81	22.69
BB	8	2.24
BC	62	17.37
CC	194	54.34

No: Number of animals

Conclusion

Using different genetic markers in the MHC-B region, it was possible to categorize MHC haplotypes in a Brown Leghorn line and evidence an effect of the MHC on the survival from MDV infection. Further analysis should estimate the potential interest of modifying the MHC genotype distribution and explore further polymorphisms associated with resistance mechanisms using a survival analysis associated with (visceral) lesion recording. Outcomes of such survival analysis can complement epidemiological studies aiming at modeling MDV infection (Atkins et al. (2011)) by providing genetic parameters for time to first symptoms or to death and estimating the risk of an individual to die at a given time. All together, genetic and epidemiological studies could be performed on vaccinated and non vaccinated birds to advance a global strategy and more integrated control of the disease.

Survival analysis. Whereas there was no significant difference in survival between the birds dying with or without nerve lesions, there was a significant difference between the birds dying with or without visceral lesions (Figure 2), evidencing the importance of these organs in the outcome of the disease. Animals without visceral lesion (but possibly with nerve lesions) were dying much less and much later and slower. Heritability of survival time was estimated to be 0.41, indicating potential for genetic improvement. Mortality to MDV has been scarcely analysed using survival analysis (Pinard et al. (1993); Weigend et al. (2001)), most studies using end-point analysis and, to our knowledge, never considering the type of lesion occurred by the disease. Although the mortality is very high in this population (88.6%), this survival analysis is the only type of analysis allowing to consider all data at any time point and to discriminate different levels of resistance, as illustrated by the different groups of birds with or without visceral lesions. As shown by recent MDV outbreaks, individual variability of response to very virulent MDV may express itself through different kinetics and different postmortem lesions (Gong et al. (2013)). Assuming that these reflect different resistance mechanisms, it is highly relevant for further search of genetic markers to consider recording lesions and applying systematically a survival analysis and not analyzing solely mortality.

Table 4. Mean ages at first symptom (1SYMPT), time to death (D-S) and death (DEATH) from MDV per MHC genotype (Geno).

Geno	1SYMPT	D-S	DEATH
AC	45.60 ^b	5.83 ^b	51.43 ^b
BC	40.52 ^a	5.70 ^b	47.69 ^a
CC	45.81 ^b	7.26 ^a	53.15 ^b

^aSYMPT, D-S and DEATH: in days

D-S = Age at death (DEATH)- Age at first symptom (1SYMPT)

Within a column, means without a common superscript differ (P < 0.05)

Acknowledgments

The authors thank J.P. Picault and ANSES (Ploufragan, France) for providing the MDV challenge facilities and advice and V. Ducrocq (INRA, UMR1313 GABI, Jouy-en-Josas, France) for helpful advice in using the survival analysis.

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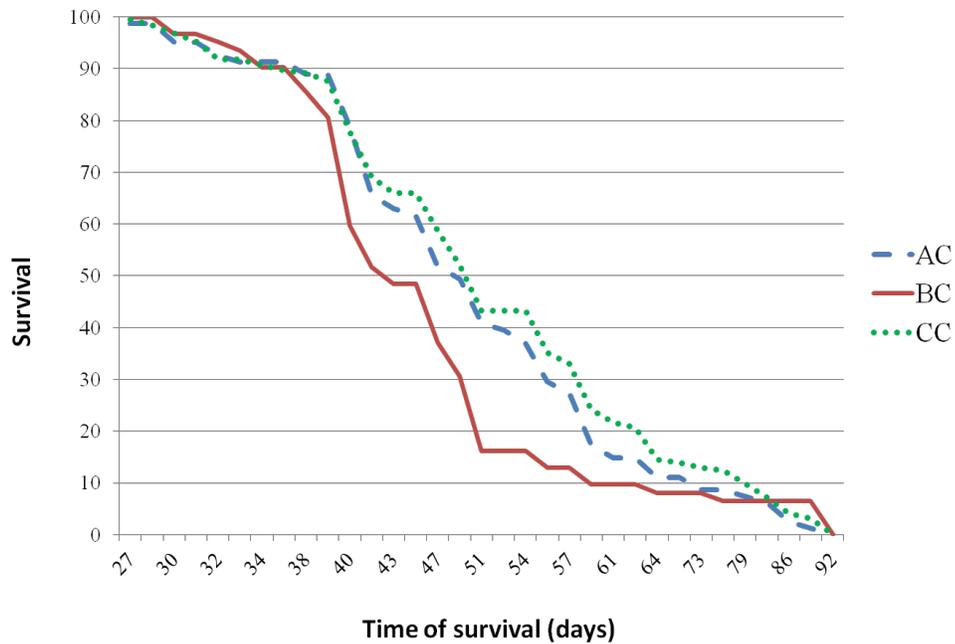


Figure 1: Survival curves per MHC genotype (AC, BC and CC).

Table 3. Primers used for MHC genotyping : name, sequence, genomic position and strand (galGal4 reference assembly).

Name	Sequence	Position	Strand
<i>LEI0258_F (HEX)</i>	HEX-CACGCAGCAGAACTTGGTAAGG	chr16:131129-131150	-
<i>LEI0258_R</i>	AGCTGTGCTCAGTCCTCAGTGC	chr16:130782-130803	+
<i>GAB0001_F (FAM)</i>	FAM-CATTCCCCATTAACGCACTC	chr16:82726-82745	-
<i>GAB0001_R</i>	AGAGTGTTGGGGTAGCCGAG	chr16:82573-82592	+
<i>GAB0002_F (FAM)</i>	FAM-CAGGGGGTCTTCAGACAAAG	chr16:186871-186890	+
<i>GAB0002_R</i>	CTTCGAGGAGCAAGAAGAG	chr16:187204-187223	-

Figure 2: Survival curves per type of lesions, visceral lesions (VL) or nerve lesions (NL).

