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Estimation of genetic parameters for resistance to gastro-intestinal nematodes in pure blood Arabian horses

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ABSTRACT

Equine internal parasites, mostly cyathostomins, affect both horse welfare and performance. The appearance of anthelmintic-resistant parasites creates a pressing need for optimising drenching schemes. This optimization may be achieved by identifying genetic markers associated with host susceptibility to infection and then to drench carriers of these markers. The aim of our study was to characterise the genetics of horse resistance to strongyle infection by estimating heritability of this trait in an Arabian pure blood population. A population of 789 Arabian pure blood horses from the Michałów stud farm, Poland were measured for strongyle egg excretion twice a year, over 8 years. Low repeatability values were found for faecal egg counts. Our analyses showed that less than 10% of the observed variation for strongyle faecal egg counts in this population had a genetic origin. However, additional analyses highlighted an age-dependent increase in heritability which was 0.04 (±0.02) in young horses (up to 3 years of age) but 0.21 (±0.04) in older ones. These results suggest that a significant part of the inter-individual variation has a genetic origin. This paves the way to a genomic dissection of horse-nematode interactions which might provide predictive markers of susceptibility, allowing individualised drenching schemes.

1. Introduction

Gastro-intestinal nematodes impair both horse health and welfare, representing an important economic burden for horse breeders. Among the species of interest, digestive strongyles are responsible for growth retardation in young horses and unthriftiness in both young and adult horses (Taylor et al., 2007). This family can be separated into two subfamilies (Lichtenfels et al., 2008): Cyathostominae (common name cyathostomins, also known as "small strongyles") and Strongylidae (common name strongylins or large strongyles). Young horses between 1 and 5 years of age (Kornaś et al., 2010; Wood et al., 2012) excrete more eggs, and a lifelong susceptibility to potential clinical disease is usually reported (Matthews et al., 2004; Corning, 2009).

Over the past decades, management of these parasites has relied primarily on the use of anthelmintic treatments. It has now become apparent that drug-resistant populations of cyathostomins have become widespread throughout the world due to the application of systematic, frequent drenching systems (von Samson-Himmelstjerna, 2012; Peregrine et al., 2014). Their pathogenic potential in conjunction with their extensive drug resistance has resulted in their ranking as one of the top 10 pathogens of equids by the French Public Health Institute (Anonymous, 2012).

One obvious strategy for limiting the rise of drug-resistant populations is to optimise drenching schemes by only targeting the horses in need of treatment (Nielsen et al., 2014). Such a strategy relies heavily on using indicators of infection that actually estimate the level of infection. To date, no better indicator has been proposed than a faecal egg count (FEC), i.e. counting the number of parasite eggs which appear in horse faeces. Also, the epidemiological features of horse infection suggest an overdispersion of faecal egg output, with 10–15% of horses excreting 80% of the eggs (Relf et al., 2013, 2014). Therefore, a genetic component of resistance to gastro-intestinal nematodes might play a role, as has already been

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demonstrated in small ruminants (Bishop et al., 2004; Kemper et al., 2011). In sheep, this genetic variability has been exploited for breeding purposes (Kemper et al., 2011; Sallé et al., 2012). Deciphering the genetic architecture of resistance to gastro-intestinal nematodes in horses may enable us to identify genetic markers of susceptibility to nematodes, and thus target horses in need of anthelmintic treatment.

A herd of pure blood Arabian horses with a recorded pedigree were measured for faecal production of gastro-intestinal nematode eggs. The aim of the study was to quantify the genetic component of susceptibility to gastro-intestinal nematodes in this pure blood Arabian horse population.

2. Materials and methods

2.1. Parasitological measurements

Data collection was conducted from 1999 to 2008 (except in 2003 and in 2007) from pure blood Arabian Horses in the Michałòw stud farm in southern Poland. These pure blood Arabian horses descended from ancestors imported to Poland during the 17th, 18th and 19th centuries (Chmiel et al., 1999). In reference to parasite control programs, horses were dewormed twice per year before and after the pasture season, in April and October, mostly with ivermectin, and no treatment was given during the pasture season.

Each year of the study, faecal samples were collected twice during the pasture season which began, depending on weather conditions, at the end of April and ended at the beginning of November. Nematode eggs were counted using a modified McMaster technique (Raynaud, 1970) in which each egg counted represented 50 eggs/g of faeces. Strongyle egg counts (SECs) which included both Cyathostominae and Strongylidae, were recorded. A previous epidemiological study had already demonstrated through larval cultures that cyathostomins make up the bulk of strongyle species in horses (Kornaś et al., 2007).

In total, 768 horses (269 males and 499 females) were sampled and resulted in a total of 2,691 records available for analysis (543 and 2,148 from males and females, respectively), which were collected in April (before the spring drenching, $n = 1{,}384$) and August ($n = 1{,}307$). On average, each horse was recorded three times in the timeframe considered (with a maximum of 14 occurrences), while for each time point considered, 179 SEC data were available on average. No geldings were present in the dataset.

2.2. Pedigree information

Heritability is the component of the observed variation that is due to additive genetic variance (Falconer and Mackay, 1996). It is estimated from the degree of phenotypic resemblance between relatives (Falconer and Mackay, 1996). This information is contained in a pedigree file that lists the parents of each individual. The more records in the pedigree, the more relatives can be identified and the better the heritability estimate. In this case, every sampled horse had a recorded mother and father so that a complete pedigree could be built. The stallions and mares which were not sampled had no pedigree information provided and were therefore considered to be ancestors, i.e. individuals with unknown parents. In the end, the pedigree file contained 945 individuals, 61 stallions and 292 mares. Among these, 17 stallions and 204 mares had at least one record in the data file. Other individuals with no phenotype contributed to estimating the genetic relationship within the whole population. However, additional pedigree information from the Michałòw stud farm, i.e. the entire family tree for every sampled individual, could not be obtained.

2.3. Data analysis

2.3.1. Variables considered

SEC data distribution was checked using the Shapiro–Wilk test implemented in R software (R Development Core Team, 2008. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria).The overdispersion of measured FECs was corrected by applying a ln(X + 10) transformation. The transformed trait was subsequently denoted ln(SEC) and used for analysis.

Due to the high number of zeros, FEC data were also considered as a binary trait (denoted Sbin), taking the value of 1 if the horse was infected and 0 if not. A limited set, comprising data recorded on individuals up to 3 years old, was also prepared to investigate age-related variation of heritability.

2.3.2. Model

Transformed continuous FECs were analysed using a linear mixed model, and binary infection statuses were analysed using a threshold model. The model was the following:

$$Y_{ijkl} = \mu + \mathrm{YM}_j + \mathrm{AGE}_k + \mathrm{SEX}_l + \mathrm{BY}_i + \mathrm{WY}_{ij} + \mathrm{A}_i + e_{ijkl} \quad (1)$$

where Y_{ijkl} is the transformed continuous FEC or the underlying variable of the threshold model for the binary infection status; μ is the population mean; YM_j is a fixed effect of year by month interaction (2 months repeated over 8 years); AGE_k is the age at sampling in years (horses of 2 and 3 years of age were grouped into one category; horses older than 15 years were grouped into the final category; nine different levels were available in total); SEX_l is the sex of the horses, being either stallions or mares; BY_i is the random permanent environmental effect of animal i over the years; WY_{ij} is the random permanent environmental effect of animal i over the months within year j; A_i is the random additive genetic effect; e_{ijkl} is the random residual effect.

2.3.3. Bivariate analyses

By applying this model to our data, the heritability of each trait was estimated, using both the whole dataset and the subset of data recorded for young horses, i.e. up to 3 years old.

To assess the extent to which two traits possessed a similar genetic architecture, pair-wise analyses between the two defined traits, i.e. SEC considered as a continuous or a binary trait, were performed to estimate additive genetic correlations (r_g). Analyses were carried out using the whole dataset or the subset of data recorded in young horses. Immunity takes some time to develop. Therefore, a bivariate analysis between records of young and adult horses was performed to assess whether the genetic architecture of nematode egg excretion differed between these two age groups.

The phenotypic correlation (r_p) between traits X and Y was subsequently derived as (Falconer and Mackay, 1996):

$$r_p = \frac{(r_g \cdot \sigma_{aX} \cdot \sigma_{aY} + r_e \cdot \sigma_{eX} \cdot \sigma_{eY} + r_r \cdot \sigma_{rY})}{\sqrt{\sigma^2_{aX} + \sigma^2_{eX}} \cdot \sqrt{\sigma^2_{aY} + \sigma^2_{eY}}},$$

where r_g, r_e and r_r are the additive genetic, random environmental and residual correlations, σ^2_{aX}, σ^2_{aY}, σ^2_{eX}, σ^2_{eY}, σ^2_{rX}, σ^2_{rY}, are the additive genetic, random environmental and residual S.D.s for traits X and Y, respectively.

2.3.4. Estimation methods

Estimation of genetic parameters was performed using a Bayesian framework implemented in TM (Threshold Model) software, freely available at http://snp.toulouse.inra.fr/~alegarra/. A chain of 500,000 iterations was used and 20% of the estimates were discarded in a burn-in procedure. Estimates for the fixed and random effects were saved every100 iterations from the a posteriori