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Short communication: Heritability estimates for susceptibility to *Mycobacterium avium* ssp. *paratuberculosis* infection in Chinese Holstein cattle

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ABSTRACT

Paratuberculosis in ruminants, which is caused by *Mycobacterium avium* ssp. *paratuberculosis* (MAP), is a contagious, chronic enteric disease associated with economic losses, animal welfare, and health implications in dairy cattle production. In this study, we estimated the variance components and heritability of susceptibility to MAP infection in Chinese Holstein cattle. We collected 4,937 serum samples from cows in 7 dairy herds in the Beijing region of China and used the ELISA test to detect antibodies to MAP. Three statistical models were implemented to estimate heritabilities: (1) a linear model (ELISA sample-to-positive ratios as a continuous trait); (2) a binary threshold model (positive/negative from ELISA results); and (3) an ordered threshold model (ELISA results as an ordered categorical model with categories 1 to 5 corresponding to negative, uncertain, mildly positive, intermediate positive, and strongly positive). The heritability estimates ranged from 0.0389 to 0.1069, indicating that genetic factors affect MAP infection susceptibility in Chinese Holstein cattle.

Key words: paratuberculosis, Johne's disease, heritability, Chinese Holstein

Short Communication

Paratuberculosis, or Johne's disease (**JD**) as it is commonly known, is caused by *Mycobacterium avium* ssp. *paratuberculosis* (**MAP**) and is a contagious, chronic, enteric disease of ruminants (Clarke, 1997). Animals suffering from JD show several classic clinical signs including diarrhea and weight loss and they eventually die. Ten percent of infected cows die of this disease every year, and the remaining 90% of diseased cows are slaughtered (Chen, 2016). *Mycobacterium avium* ssp. *paratuberculosis* can hide in many substrates such as colostrum, milk, and feces, from where it can be transmitted to susceptible individuals (Nielsen et al., 2008), with the major route being the fecal-oral route (Lombard, 2011). Since 2000, JD has been reported around the world with an average herd prevalence (the proportion of herds with at least one JD case) between 30 and 50% (Boelaert et al., 2000; Muskens et al., 2000; Tiwari et al., 2006; NAHMS, 2007; Haghkhah et al., 2008; Defra, 2009; Good et al., 2009; Nielsen, 2009a,b,c). According to recent reports, herd prevalence has reached 91.1% in the United States (Lombard et al., 2013) and 65.4% in Egypt (Amin et al., 2015). The associated costs of JD, such as decreased reproductive and productive efficiency and the need for diagnostic testing, have resulted in JD affecting the global dairy industry severely (Ott et al., 1999). Unfortunately, because no effective cure or vaccines for JD exist, the only way to decrease its prevalence is to implement control programs such as those described by Ferrouillet et al. (2009). The main steps of control programs include identifying positive cows using ELISA or other detection methods, isolating them to eliminate transmission routes, and finally eliminating positive cows that show clinical symptoms.

Selection for disease-resistant animals could produce offspring with increased average ability to resist MAP infection; thus, genetic selection for animals with JD resistance would be an effective means to control JD. Several early studies performed on Holstein and Jersey cattle that estimated the heritability of infection with MAP reported estimates ranging from 0.031 to 0.283 (Koets et al., 2000; Mortensen et al., 2004; Gonda et al., 2006; Hinger et al., 2008; Attalla et al., 2010; Berry et al., 2010; van Hulzen et al., 2011; Küpper et al., 2012; Shook et al., 2012; Zare et al., 2014). Despite variable results, these previous studies show that MAP-infection resistance in cattle has a genetic background.

Chinese Holstein cattle are the result of cross breeding between Chinese Yellow cattle and European Holsteins

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over the past 100 yr. Foreign Holstein bulls, semen, and embryos, mainly from the United States, and a few from Canada and Europe, have been continuously imported, and these have been used directly for AI or for crossing with Chinese Holstein cows through planned mating to generate breeding bulls (Sun et al., 2009). According to recent investigations on MAP in large-scale dairy farms in some Chinese provinces, herd-level prevalence of MAP infection has reached 100% (Sun et al., 2015; Cui et al., 2016) and within-herd prevalence has also gradually increased. To the best of our knowledge, no systematic study to analyze the genetics of susceptibility to MAP infection in Chinese Holstein cattle has been undertaken. To genetically improve resistance to MAP infection, implementation of a comprehensive genetic evaluation is a crucial step. Therefore, the objective of this study was to estimate the heritability of susceptibility to MAP infection based on antibody titers from Chinese Holstein cattle, and to use the data in the context of dairy cattle selection.

The protocols for collecting blood samples from the experimental animals were reviewed and approved by the Institutional Animal Care and Use Committee at China Agricultural University (Permit Number DK996). All the experiments were performed in accordance with the relevant approved guidelines and regulations. In total, 8,214 Chinese Holstein cows from 7 herds at the Beijing Sanyuan Dairy Farm Center were sampled, 4,937 of which were ≤24 mo of age and used for heritability estimation. All cows were fed under the same feeding and management system, and regular quarantine inspections of the herds were conducted so that these 7 herds represented the situation of JD infection status of dairy herds in the Beijing region. Although all 7 herds belong to the Beijing Sanyuan Dairy Farm Centre, there was no movement of animals between the herds because of the strict management system based on regular quarantine inspections twice annually. In addition, there were no records of other diseases such as tuberculosis or subclinical mastitis in the cows included in this study. The cows belonged to 436 sire families with an average of 11.3 daughters per sire. Pedigree relationships for the 4,937 cows were traced back 5 generations. Blood samples (500 μL) were collected from the caudal vein of each cow during the regular quarantine inspection of the farms in September 2014. All cows within a herd were sampled on the same day. Serum extracted from blood samples was stored at 4°C until testing, which took place within 5 d of collection. With the ELISA method, the antibody levels in the serum samples were determined using the *Mycobacterium paratuberculosis* Antibody Test Kit (Idexx Laboratories Inc., Westbrook, ME) following the manufacturer's instructions. The MAP status of an animal was expressed as the sample-to-positive (**S/P**) ratio multiplied by 100: S/P ratio = 100 × [(optical density (OD) value of the sample – OD of the negative control)/(OD of a positive sample – OD of the negative control)], where S/P ≤ 0.45 is negative; 0.45 < S/P < 0.55 is uncertain; 0.55 ≤ S/P < 1 is mildly positive; 1 ≥ S/P < 2 is intermediate positive; and S/P >2 is strongly positive. Parity levels were from 0 (nulliparous) to 4 (≥ fourth parity). The ages of the cows sampled ranged from 25 to 162 mo. Ages were grouped in 17 levels by 6-mo intervals (all cows older than 120 mo were in level 21), and 77.17% of individuals were between 25 and 60 mo old. Three traits were defined for heritability estimation according to the ELISA results: (1) ELISA S/P were taken as a continuous trait; (2) ELISA results were taken as a binary trait (0 = negative, 1 = positive), where mildly positive, intermediate positive, and strongly positive ELISA results were considered ELISA positive (77 uncertain ELISA results were excluded); and (3) ELISA results were taken as an ordered categorical trait with categories 1 to 5 corresponding to negative, uncertain, mildly positive, intermediate positive, and strongly positive, respectively.

We used an animal genetic model and 3 statistical models: a linear model, a binary threshold model, and an ordered threshold model to estimate the variance components. Because of non-normality, log-transformed ELISA S/P ratios were used in the linear model [ELISA = $\log_{10}$(ELISA + 0.01); 0.01 was added to avoid log(0)]. Before estimating variance components, a preliminary analysis of the fixed effects including herd and parity was conducted using the different models. The fixed effects for herd and parity showed significant ($P < 0.01$) effects in all 3 models.

The following linear model was used for the analysis of log-transformed ELISA S/P ratios:

$$y_{ijkn} = p_i + h_j + a_k + e_{ijkn},$$

where y_{ijkn} is the transformed ELISA S/P ratio, p_i is the fixed effect of parity, h_j is the fixed effect of herd, a_k is the additive genetic effect, and e_{ijkn} is the residual. Random effects were assumed to be normally distributed:

$$a \sim N\left(0, \mathbf{A}\sigma_a^2\right),$$

$$e \sim N\left(0, \mathbf{I}\sigma_e^2\right),$$

where $\mathbf{A}$ is the numerator relationship matrix, σ_a^2 represents the individual additive variance, $\mathbf{I}$ is an identity matrix, and σ_e^2 represents the residual variance. Variance components were estimated by the DMUAI mod-