The EDN2 rs110287192 gene polymorphism is associated with paratuberculosis susceptibility in multibreed cattle population

Mehmet Ulaş Çınar1,2,*, Bilal Akyüz3, Korhan Arslan3, Stephen N. White2,4,5, Holly L. Neibergs5,6, Kadir Semih Gümüşsoy7

1 Department of Animal Science, Faculty of Agriculture, Erciyes University, Kayseri, Turkey, 2 Department of Veterinary Microbiology & Pathology, Washington State University, Pullman, WA, United States of America, 3 Department of Genetics, Faculty of Veterinary Medicine, Erciyes University, Kayseri, Turkey, 4 Animal Disease Research Unit, Agricultural Research Service, U.S. Department of Agriculture, Pullman, WA, United States of America, 5 Center for Reproductive Biology, Washington State University, Pullman, WA, United States of America, 6 Department of Animal Science, Washington State University, Pullman, WA, United States of America, 7 Department of Microbiology, Erciyes University, Faculty of Veterinary Medicine, Kayseri, Turkey

* mucinar@erciyes.edu.tr

Abstract

Paratuberculosis (pTB), also known as Johne's disease (JD), is a contagious, chronic, and granulomatous inflammatory disease of the intestines of ruminants which is caused by Mycobacterium avium subsp. paratuberculosis (MAP) infection, resulting in billions of dollars in economic losses worldwide. Since, currently, no effective cure is available for MAP infection, it is important to explore the genetic variants that affect the host MAP susceptibility. The aim of this study was to analyze a potential association between EDN2 synonymous gene mutations (rs110287192, rs109651404 and rs136707411), that modifies susceptibility to pTB. EDN2 rs110287192 mutation showed a significant association with bovine pTB (adj. p < 0.05). For rs110287192 locus, the odd ratios for GG and TG genotypes versus TT genotypes were 1.73; (95% CI = 0.34–8.59) and 0.53 (95% CI = 0.12–2.37) respectively, which indicated that proportion of TG heterozygotes were significantly higher in control animals as compared to pTB animals. On the other hand, while rs136707411 mutation showed a suggestive association with pTB status in the examined cattle population (nominal p < 0.05); no association was detected between rs109651404 genotypes and pTB status. Selecting animals against rs110287192-GG genotype may decrease the risk of pTB in cattle of the Bos taurus taurus subspecies.
Paratuberculosis (pTB), or Johne's Disease, is a chronic disease affecting ruminant livestock, and is caused by intestinal infection with Mycobacterium avium subsp. paratuberculosis (MAP) [1]. MAP is a Gram-positive intracellular pathogen which is dependent on mycobactin, and thus unable to replicate in the environment [2]. MAP's ability to infect other animals through indirect contact is facilitated by prolonged survival times. For instance, MAP remained viable and was transmitted for up to 55 weeks in a shaded, outdoor area in Australia Whittington et al. [3]. Animals are usually MAP infected at a young age and are generally believed to undergo an extended latent period of chronic infection [4]. pTB begins as a localized infection that may become systemic and often results in chronic granulomatous enteritis leading eventually to weight loss, (diarrhea in some species) and death [1]. Therefore, pTB causes considerable economic losses to livestock farmers, particularly in dairy cows and beef cattle. A recent study estimated annual cost caused by pTB in the United States to be $20.80 per dairy cow [5] and this value may estimate at up to $72.5 per cow per year in Netherlands [6]. Although, data to estimate losses from pTB in beef herds are limited, Bhattarai et al. [7] reported an annual average loss of $276 (95% CR: $149–$478) per infected beef cattle based on survey responses. Beside direct losses as described above, indirect losses due to national and international trade restrictions and public health concerns may arise. Controversy remains as to the causation between Crohn's disease (CD) in humans and exposure to pTB, although some experiments have already shown that there is a link between pTB and CD in humans [8,9]. Despite the application of several control strategies, such as testing, vaccination and culling to reduce pTB transmission between herds, many countries continue to face challenges in controlling pTB [1]. Therefore, understanding the genetic basis of pTB susceptibility could be an alternative method for reducing the disease and selecting cattle for enhanced resistance against pTB [10,11].

Endothelins (EDN), with three isoforms of 21-residue peptides (EDN1, EDN2, EDN3), two G-protein coupled receptors (ETA and ETB), and two endothelin-converting enzymes (ECE-1 and ECE-2), are vasoconstrictor peptides [12]. The EDN are involved in the regulation of many physiological processes, such as cardiovascular development and function, craniofacial development, blood pressure regulation, renal water and sodium excretion, neurotransmission, ovulation, and proliferation, migration and differentiation of cranial, cardiac, trunk, sacral and neural crest cells [12,13]. Among EDN genes, EDN2 has been studied in terms of ovarian research especially due to its roles in steroidogenesis and corpus luteum formation in human, model organisms and in livestock [14]. Takizawa et al. [15] investigated the EDN2 expression in mice and revealed that EDN2 mRNA was abundant in epithelial cells of the mucosal layer in the intestinal tract which may be associated with modulation of the mucosal defense by triggering immune cells. In livestock, EDN2 has been investigated for its corpus luteum formation in cattle [16] and mRNA expression profiling in chicken tissues [17]. In addition, Settles et al. [18] and Neibergs et al. [19] reported EDN2 as a strong functional and positional candidate gene for pTB susceptibility in Holstein cattle according to GWAS study. EDN2 locus was identified with genome-wide significant level of association to the presence of MAP in tissue and both tissue and feces, respectively [18]. Three EDN2 synonymous mutations on bovine chromosome 3 (BTA3), named rs110287192, rs109651404 and rs109490418 were patented for being associated with pTB susceptibility in Holstein breed cattle [20].

This study aimed to examine the association between EDN2 SNPs rs110287192, rs109651404, rs109490418 and pTB susceptibility in a Holstein population reared in Turkey and in Turkish indigenous cattle crossbreds (East Anatolian Red Cattle and Native Black Cattle). Genotyping experiments for three EDN2 SNPs were conducted and the relationship with pTB susceptibility in three cattle populations was evaluated.
Materials and methods

Sample collection

We undertook this case-control study between June 2014 and August 2014. All experimental procedures were performed in accordance with the guidelines of the Local Ethics Committee for Animal Experiments at Erciyes University (#14/77-09.04.2014). All samples were received for confirmation of a clinical suspicion of pTB in the herd and had no further follow-up. Cattle were classified as infected (cases) if they were positive for blood serum enzyme-linked immunosorbent assay (ELISA). Animals that were both clinically negative and serologically negative were considered healthy (controls). Further details regarding sample collection and ELISA diagnostic tests have been published elsewhere [21]. The study population was found to consist of 68 infected and 750 healthy animals. Briefly, blood samples were collected from cattle at two to three years of age including East Anatolian Red crossbred (n = 288), Anatolian Black crossbred (n = 112) and Holstein (n = 418) breeds from the Kayseri province and its vicinity in Turkey. Animals included in the present study were housed in similar environmental conditions and not vaccinated for pTB. Blood samples were used for the isolation of genomic DNA for genotyping, and serum samples were used for detection of MAP antibodies by ELISA.

SNP selection

Three SNP selection methods were followed in the present study. First, we obtained genotype data of U.S. Holsteins from the existing literature on the association of *EDN2* gene polymorphisms with MAP tissue infection and pTB susceptibility [19]. Second, rs110287192–g.104700352T>G in 5′ UTR variant, rs109651404–g.104689861G>A intergenic variant and rs109490418–g.104706758G>A in 3′ UTR variant mutations were patented by Neibergs et al. patent# US20140283151 [20] for selective breeding to produce offspring having at least one of susceptibility, resistance or tolerance to pTB. Third, since assay design for the patented rs109490418 mutation failed due to too many variants in the immediate vicinity, another mutation which is linked (D’ = 0.88, r² = 0.28) and 6166 bp downstream rs136707411–g.104700592G>A in 5′ UTR variant region was selected for genotyping. The susceptible alleles for rs11028192 and rs109651404 were previously reported as G and A, respectively [19].

Genotyping

Genomic DNA was extracted from whole blood using a standard phenol–chloroform extraction procedure. DNA concentration of the samples were quantified by Nano Drop (NanoDrop, Thermo Fisher Scientific, Waltham, MA, USA), diluted to 50 ng/μl and stored at -20°C until used. rs110287192, rs109651404 and rs136707411 SNPs of *EDN2* were genotyped using the TaqMan allelic discrimination method, which determines variants of single nucleic acid sequence. Since current SNPs have not been genotyped by using any other method, the custom TaqMan chemistry was selected as cost and time effective genotyping method. Using two primer/probe pairs in each reaction allows genotyping of the two possible variants at the single nucleotide polymorphism in a target sequence template. Details of assay IDs, primer and probe sequences were given in the Table in S1 Table. The genotyping PCR reaction was performed by adding 2 μl of genomic DNA template, 5 μl of genotyping master mix (Thermo Fisher Scientific, Waltham, MA, USA), 0.5 μl of the genotyping custom-made assay mix (probes and primers) (Thermo Fisher Scientific, Waltham, MA, USA) and 2.5 μl of DNAase-free water. Two negative controls were included on each plate. For the negative controls, 2.5 μl of DNAase-free water was added to each reaction plate instead of genomic DNA for the sample. The cycling parameters were as follows: first, denaturation was done at 95°C for 10 min,
followed by 40 cycles of denaturation at 95˚C for 15 s, annealing and extension at 60˚C for 60 s. The PCR was performed in a StepOne Real-Time PCR System (Thermo Fisher Scientific, Waltham, MA, USA).

**Statistical analysis**

An online software (http://www.husdyr.kvl.dk/kc/popgen/genetik/applets/kitest.htm) was used to analyze the Hardy-Weinberg equilibrium (HWE) and allele frequency for each SNP and statistical significance was defined as \( p < 0.05 \). Data were analyzed using SAS 9.2 software (SAS Institute Inc., Cary, NC, USA). Additive genetic model was used for statistical analysis. The univariable analysis for logistic regression considered the infection status as a categorical response variable (yes/no), and SNPs (all three SNPs have three genotypes, therefore respective loci have three levels), breed (three groups i.e. two indigenous crossbred and Holstein) and sex (male and female) were included as possible explanatory variables. Genotypes were considered as ordinal variables and as class variables with the major homozygous genotype deemed as baseline. Data were analyzed using PROC LOGISTIC procedure and odds ratios (OR) with 95% confidential intervals (CIs) were calculated. Bonferroni correction (based on the total number of markers tested) was used for multiple comparisons correction, and statistical significance was defined as \( p < 0.05 \).

**Results**

A total of 818 animals met the inclusion criteria and were included in the study to be genotyped, of which 68 had a diagnosis of pTB according to ELISA OD values (\( \geq 1.0 \)) were subjected to association analysis and were compared to 750 age-matched healthy controls. The genotyping success rates were 97%, 94% and 90% for rs110287192, rs109651404 and rs136707411, respectively and the consensus rate (on the basis of 5% duplicates) was 100% for DNA isolated from whole blood. Although, the genotype frequencies of rs109651404 (\( \chi^2 = 0.0042 \) for case and \( \chi^2 = 1.07 \) for control) and rs136707411 (\( \chi^2 = 0.02 \) for case and \( \chi^2 = 2.98 \) for control) SNPs were in accordance with the Hardy–Weinberg equilibrium in both control group and case, genotype frequencies of rs110287192 SNP was significantly deviated from Hardy–Weinberg equilibrium (\( \chi^2 = 35.17 \) for case and \( \chi^2 = 36.31 \) for control) due to a deficit of homozygous genotypes (TT) of the most frequent allele. The distribution of the bovine EDN2 rs110287192, rs109651404 and rs136707411 genotypes and allele frequencies in the study population are shown in Table 1.

Genotypic association analysis of all three EDN2 polymorphisms with pTB are shown in Table 2. A significant association with the pTB was found for the EDN2 rs110287192 variant

| SNP              | Genotypes (%) | Allele (%) | \( \chi^2 \) (\( \alpha = 0.05 \), df = 1) |
|------------------|---------------|------------|-------------------------------------|
| rs110287192 n = 796 | TT 54 (6.78)  | TG 463 (58.17)  | GG 279 (35.05)  | T+ 510 (64)  | G 287 (36)  | 55.31* |
| rs109651404 n = 769 | GG 323 (42)  | GA 341 (44.3)  | AA 105 (13.7)  | G+ 500 (65) | A 269 (35) | 0.97 |
| rs136707411 n = 751 | AA 291 (38.7)  | GA 330 (43.9)  | GG 130 (17.4)  | A 450 (60) | G 301 (40) | 4.66 |

* \( p \leq 0.05 \) indicates statistical significance
** Favorable allele in previous studies

https://doi.org/10.1371/journal.pone.0238631.t001
Table 2. Univariate logistic regression analysis of studied bovine EDN2 variants and independent factors associated with pTB cases and controls.

| SNP          | Genotype | Phenotype frequency | Nominal p-value | Adjusted p-value* | Fixed factors | OR (95% CI) |
|--------------|----------|---------------------|-----------------|-------------------|---------------|-------------|
|              |          | Case (%)            | Control (%)     |                   | Sex           | Breed       |
| rs110287192  | TT       | 2 (3.70)            | 52 (96.3)       | 0.013             | NS            | 1.00        |
|              | TG       | 58 (12.53)          | 405 (87.47)     |                   | *             | 0.53 (0.12–2.37) |
|              | GG       | 8 (2.87)            | 271 (97.13)     |                   | 1.73 (0.34–8.59) |
| rs109651404  | GG       | 31 (9.6)            | 292 (90.4)      | 0.99              | NS            | 1.00        |
|              | GA       | 30 (2.33)           | 311 (91.2)      |                   | NS            | 0.98 (0.32–2.97) |
|              | AA       | 7 (6.67)            | 98 (93.33)      |                   | 0.76 (0.18–3.16) |
| rs136707411  | GG       | 25 (19.23)          | 105 (80.77)     | 0.023             | NS            | 1.00        |
|              | GA       | 32 (9.42)           | 298 (90.58)     |                   | NS            | 1.66 (0.92–2.98) |
|              | AA       | 11 (3.78)           | 280 (96.22)     |                   | 2.94 (1.34–6.46) |

Abbreviations: OR: odds ratio; 95% CI: 95% confidence interval

* p ≤ 0.05 indicates statistical significance
** p < 0.01 indicates statistical significance
p-value was adjusted by Bonferroni correction; NS: not significant p > 0.05

When the TT genotype was used as a reference, while genotype GG alone (OR = 1.73; 95% CI = 0.34–8.59; adj. p < 0.05) were significantly associated with a higher risk of pTB, genotype TG was associated with lower risk of pTB (OR = 0.53; 95% CI = 0.12–2.37; adj. p < 0.05) (Table 2). This association remained significant after Bonferroni correction for multiple tests (Bonferroni-corrected significance level for three SNPs is 0.05/3 = 0.016).

In addition, we observed suggestive association between the EDN2 rs136707411 and increased pTB risk (nominal p = 0.023; Table 2). The association did not remain significant after Bonferroni correction for multiple tests (Table 2). At the EDN2 rs136707411 locus, the OR of GA genotype versus GG genotype was 1.66 (95% CI = 0.92–2.98; nominal p < 0.05) and AA genotype versus GG genotype was 2.94 (95% CI = 1.34–6.46; nominal p < 0.05) which revealed that genotypes GA and AA increases the risk of pTB compared to genotype GG (Table 2). No genotype of EDN2 rs109651404 were found to be significant associated with pTB (all p > 0.05).

Discussion

Paratuberculosis (Johne’s disease) causes a chronic diarrhea characterized by a malabsorption syndrome. The lack of absorption of nutrients in the gastrointestinal tract leads to malnutrition, muscular wasting and eventually death which results in significant economic impact worldwide [22]. Crohn’s disease, a granulomatous enteritis in humans that can persist for decades, has clinical similarities with pTB in ruminants. Due to the clinical similarities between pTB and Crohn’s disease, the role of MAP in Crohn’s disease has been of interest [8]. Approximately 1.4 million people in North American are affected with Crohn’s disease [9] and its prevalence is rapidly increasing incidence worldwide, especially in newly industrialized countries, making Crohn’s as a global disease [23].

Therefore, eradication of pTB might be vital both for ruminant and public health. Control strategies to eradicate pTB mainly depend on: a) management strategies based on avoiding contact of susceptible young stock with infected animals, and b) testing animals with ELISA and culling infectious animals in herds [24]. Although management and testing strategies were powerful in reducing the infection, due to low specificity of ELISA tests and lack of effective vaccine, eradication of pTB has been shown to be difficult [24]. Thus, additional approaches,
such as genomic selection for cattle less susceptible to pTB to control pTB, are needed. Similar to our results, variability among cattle breeds in their susceptibility to pTB were identified in different experiments and support that selection for enhanced resistance to the disease is possible [21,25–27].

Association of bovine pTB susceptibility with EDN2 was first identified with a GWAS [18] and SNP-based gene-set enrichment analysis for MAP infection detected via tissue infection or fecal shedding by using in 245 US Holsteins [19]. In a subsequent study, the EDN2 variants rs109651404, rs110287192 and rs109490418 mutations were patented for being candidate SNPs for selection of cattle that were less susceptible to MAP infection in Holstein cows [20]. In the present experiment, rs110287192 SNP was validated as significantly associated with pTB susceptibility in a larger cattle population that consisted of Holstein and Turkish indigenous cattle crossbreds (Table 2). For the rs110287192 locus, the OR for TG genotypes versus TT genotypes was 0.53 (0.12–2.37; 95% CI) which revealed that the relative proportion TG genotypes was significantly higher in the control population than in the case population. It indicated that the TG genotype at the rs110287192 locus was associated with decreased relative risk of bovine pTB and consequently selection in favor of the TG genotype or the T allele may reduce risk of pTB in cattle (Table 2). Due to the relatedness of mycobacterial pathogens such as MAP, Mycobacterium tuberculosis and Mycobacterium bovis, loci that provide less genetic susceptibility to one pathogen might afford some protection to the other organism. In fact, loci on BTA3 where we identified association for pTB susceptibility in the current study, overlapped with loci previously reported in the literature that were associated with bovine tuberculosis susceptibility [28] and bovine respiratory disease susceptibility [29].

The literature is rather sparse for identifying an association between EDN2 variants with production or immune traits in livestock species. In cattle, pig, and laboratory animals, EDN2 acts in the regulation of steroid production of granulosa cells [14] and EDN2 mRNA expression found to be responsible for corpus luteum formation and ovulation [16,30,31]. Although EDN2 was not found to be directly associated with immune traits, knockout mice for endothelin receptor B (EDNRB) which is a G-protein-coupled receptor of EDN2, developed Hirschsprung’s disease (HSCR) [32]. This disease is characterized by a lack of ganglion cells of the colon and exhibits severe inflammation of the intestinal mucosa leading to like the clinical presentations associated with inflammatory bowel disease (IBD) [33]. IBD is a chronic inflammatory disease of the gastrointestinal tract in humans that can be divided into those with Crohn’s disease, where disease may be present throughout the GI tract and those with Ulcerative Colitis, where disease is limited to the colon. There has been speculation that Crohn’s disease may be caused by MAP as well [34].

In the present study, a strong association between a variant of EDN2, rs110287192, and pTB susceptibility in Holstein and two Turkish indigenous cattle crossbreds was demonstrated, validating, and extending the association that was previously described [18,19]. Such validation provides important support for the biological role and practical application of genomic selection for this variant [35]. Furthermore, our data also contributes to the understanding of bovine pTB and provides information that may be useful as an approach to reduce the disease through selection. Selecting against animals with the rs110287192-GG genotype may decrease the risk of pTB in Bos taurus cattle. Further analyses that are combining EDN2 genotyping and holistic expression methods through expanded sampling of other cattle breeds together with blood mRNA and serum samples for protein expression are recommended to better understand the role genomic selection could play in reducing the susceptibility to pTB in cattle.
Supporting information

S1 Table. Primer and probes, used for genotyping of EDN2 rs109651404, rs110287192 and rs136707411 SNPs. F: forward; R: reverse; * assay IDs given by prob production company.

Acknowledgments

The authors indebted to Ms. Codie Durfee for technical assistance during experiments.

Author Contributions

Conceptualization: Mehmet Ulaş Çınar, Bilal Akyüz, Kadir Semih Gümüşsoy.
Funding acquisition: Mehmet Ulaş Çınar, Bilal Akyüz, Korhan Arslan, Kadir Semih Gümüşsoy.
Methodology: Mehmet Ulaş Çınar, Bilal Akyüz, Korhan Arslan, Kadir Semih Gümüşsoy.
Project administration: Mehmet Ulaş Çınar.
Validation: Stephen N. White, Holly L. Neibergs.
Writing – original draft: Mehmet Ulaş Çınar.
Writing – review & editing: Mehmet Ulaş Çınar, Stephen N. White, Holly L. Neibergs.

References

1. Whittington R, Donat K, Weber MF, Kelton D, Nielsen SS, Eisenberg S, et al. Control of paratuberculosis: Who, why and how. A review of 48 countries. BMC Vet Res. 2019; 1–29. https://doi.org/10.1186/s12917-018-1758-8
2. Lambrecht R, Collins MT. Mycobacterium paratuberculosis. Factors that influence mycobactin dependence. Diagn Microbiol Infect Dis. 1992; 15(3): 239–246. https://doi.org/10.1016/0732-8893(92)90119-e PMID: 1582168
3. Whittington RJ, Marshall DJ, Nicholls PJ, Marshall IB, Redd acliff LA. Survival and dormancy of Mycobacterium avium subsp. paratuberculosis in the environment. Appl Environ Microbiol. 2004; 70: 2989–3004. https://doi.org/10.1128/aem.70.5.2989-3004.2004 PMID: 15128561
4. Marcé C, Ezanno P, Weber MF, Seegers H, Pfeiffer DU, Fourichon C. Invited review: Modeling within-herd transmission of Mycobacterium avium subsp. paratuberculosis in dairy cattle: A review. J Dairy Sci. 2010; 93: 4455–4470. https://doi.org/10.3168/jds.2010-3139 PMID: 20854979
5. Vertseramo Chiu LJ, Tauer LW, Al-Mamun MA, Kaniyamattam K, Smith RL, Grohn YT. An agent-based model evaluation of economic control strategies for paratuberculosis in a dairy herd. J Dairy Sci. 2018; 101: 6443–6454. https://doi.org/10.3168/jds.2017-13175 PMID: 29705432
6. Groenendaal H, Nielen M, Jalvingh AW, Horst SH, Galligan DT, Hesselink JW. A simulation of Johne’s disease control. Prev Vet Med. 2002; 54: 225–245. https://doi.org/10.1016/s0167-5877(02)00027-2 PMID: 12114011
7. Bhattarai B, Fosgate GT, Osterstock JB, Fossler CP, Park SC, Roussel AJ. Perceptions of veterinarians in bovine practice and producers with beef cow-calf operations enrolled in the US Voluntary Bovine Johne’s Disease Control Program concerning economic losses associated with Johne’s disease. Prev Vet Med. 2013; 112: 330–337. https://doi.org/10.1016/j.prevetmed.2013.08.009 PMID: 24034813
8. Timms VJ, Daskalopoulos G, Mitchell HM, Neilan BA. The Association of Mycobacterium avium subsp. paratuberculosis with Inflammatory Bowel Disease. PLoS One. 2016; 11:e0148731. https://doi.org/10.1371/journal.pone.0148731 PMID: 26849125
9. McNees AL, Markesich D, Zayyani NR, Graham DY. Mycobacterium paratuberculosis as a cause of Crohn’s disease. Expert Rev Gastroenterol Hepatol. 2015; 9: 1523–1534. https://doi.org/10.1586/17474124.2015.1039331 PMID: 26474349
10. Raadsm HW, Conington J. Breeding for disease resistance in farm animals [Internet]. Bishop SC, Axford RFE, Nicholas FW, Owen JB, editors. Wallingford: CABI; 2010. https://doi.org/10.1079/9781845935559.0000
11. Bishop SC, Woolliams JA. Genomics and disease resistance studies in livestock. Livest Sci. 2014; 166: 190–198. https://doi.org/10.1016/j.livsci.2014.04.034 PMID: 2639300

12. Davenport AP, Hyndman KA, Dhaun N, Southan C, Kohan DE, Pollock JS, et al. Endothelin. Pharmacol Rev. 2016; 68(2): 357–418. https://doi.org/10.1124/pr.115.011833 PMID: 26956245

13. Kohan DE, Rossi NF, Insoho EW, Pollock DM. Regulation of blood pressure and salt homeostasis by endothelin. Physiol Rev. 2011; 77. https://doi.org/10.1152/physrev.00060.2009 PMID: 21248162

14. Ervin JM, Schütz LF, Spicer LJ. Current status of the role of endothelins in regulating ovarian follicular function: A review. Anim Reprod Sci. 2017; 1–10. https://doi.org/10.1016/j.anireprosci.2017.09.008 PMID: 28967452

15. Takizawa S, Uchide T, Adur J, Kozakai T, Kotake-Nara E, Quan J, et al. Differential expression of endothelin-2 analogues in the mouse intestinal tract. J Mol Endocrinol. 2005; 35: 201–209. https://doi.org/10.1677/jme.1.01787 PMID: 16216902

16. Klipper E, Levit A, Mastich Y, Berisha B, Schams D, Meidan R. Induction of endothelin-2 expression by luteinizing hormone and hypoxia: Possible role in bovine corpus luteum formation. Endocrinology. 2010; 151: 1914–1922. https://doi.org/10.1210/endo.2009-0767 PMID: 20176726

17. Liu H, Luo O, Zhang J, Mo C, Wang Y, Li J. Endothelins (EDN1, EDN2, EDN3) and their receptors (EDNRA, EDNRB, EDNRB2) in chickens: Functional analysis and tissue distribution. Gen Comp Endocrinol. 2019; 283: 113231. https://doi.org/10.1016/j.ygcen.2019.113231 PMID: 31351053

18. Settles M, Zanella R, McKay SD, Schnabel RD, Taylor JF, Whitlock R, et al. A whole genome association analysis identifies loci associated with Mycobacterium avium subs. paratuberculosis infection status in US Holstein cattle. Anim Genet. 2009; 40: 655–662. https://doi.org/10.1111/j.1365-2052.2009.01896.x PMID: 19422364

19. Neibergs HL, Settles ML, Whitlock RH, Taylor JF. GSEA-SNP identifies genes associated with Johnes’ disease in cattle. Mamm Genome. 2010; 21: 419–425. https://doi.org/10.1007/s00335-010-9278-2 PMID: 20706723

20. Neibergs HL, Ricardo Z, Taylor JF, Wang Z, Scraggs E, White SN, et al. Compositions and methods for diagnosis of genetic susceptibility, resistance, or tolerance to infection by mycobacteria and bovine paratuberculosis using promoter variants of EDN2. Google patents [Preprint]. 2014 [cited 2020 April 17]. Available: https://patents.google.com/patent/US20140283151

21. Cinar MU, Hizilsoy H, Akyüz B, Arslan K, Aksel EG, Gümüşsoy KS. Polymorphisms in toll-like receptor (TLR) 1, 4, 9 and SLC11A1 genes and their association with paratuberculosis susceptibility in Holstein and indigenous crossbred cattle in Turkey. J Genet. 2018; 97: 1147–1154. Available: http://www.ncbi.nlm.nih.gov/pubmed/30555064 PMID: 30555064

22. Rathnaiah G, Zinniel DK, Bannantine JP, Stabel JR, Grohn YT, Collins MT, et al. Pathogenesis, molecular genetics, and genomics of Mycobacterium avium subs. paratuberculosis, the etiologic agent of Johnes’ disease. Front Vet Sci. 2017; 4: 187. https://doi.org/10.3389/fvets.2017.00187 PMID: 29164142

23. Schmitt H, Neufert C, Neurath MF, Atreya R. Resolution of Crohn’s disease. Semin Immunopathol. 2019; 41(6): 737–746. https://doi.org/10.1007/s00281-019-00756-1 PMID: 31552470

24. Van Hulzen KE, Koets AP, Nielen M, Heuven HCM, Van Arendonk JAM, Klinkenberg D. The effect of farm and management factors in dairy cattle in England. Prev Vet Med. 1997; 32: 176–177. https://doi.org/10.1016/S0167-5877(97)00028-7 PMID: 9443332

25. Norton S, Heuer C, Jackson R. A questionnaire-based cross-sectional study of clinical Johnes’ disease on dairy farms in New Zealand. N Z Vet J. 2009; 57: 34–43. https://doi.org/10.1080/00480169.2009.36866 PMID: 19252541

26. Sorge US, Lissimore K, Godkin A, Hendrick S, Wells S, Kelton D. Associations between paratuberculosis milk ELISA result, milk production, and breed in Canadian dairy cows. J Dairy Sci. 2011; 94: 754–761. https://doi.org/10.3168/jds.2010-3404 PMID: 21257043

27. Cetinkaya B, Erdogan HM, Morgan KL. Relationships between the presence of Johnes’ disease and farm and management factors in dairy cattle in England. Prev Vet Med. 1997; 32: 253–66. https://doi.org/10.1016/S0167-5877(97)00028-7 PMID: 9443332

28. González-Ruiz S, Strilacci MG, Durán-Aguilar M, Cantó-Alarcón GJ, Herrera-Rodríguez SE, Bagnato A, et al. Genome-wide association study in Mexican Holstein cattle reveals novel quantitative trait loci regions and confirms mapped loci for resistance to bovine tuberculosis. Animals (Basel). 2019; 9(9): 636. https://doi.org/10.3390/ani9090636 PMID: 31480266

29. Neupane M, Kiser JN, Neibergs HL. Gene set enrichment analysis of SNP data in dairy and beef cattle with bovine respiratory disease. Anim Genet. 2018; 49: 527–538. https://doi.org/10.1111/age.12718 PMID: 30229962
30. Cacioppo JA, Oh SW, Kim HY, Cho J, Lin PCP, Yanagisawa M, et al. Loss of function of endothelin-2 leads to reduced ovulation and CL formation. PLoS One. 2014; 9. https://doi.org/10.1371/journal.pone.0096115 PMID: 24763822

31. Iwai M, Hasegawa M, Taii S, Sagawa N, Nakao K, Imura H, et al. Endothelins inhibit luteinization of cultured porcine granulosa cells. Endocrinology. 1991; 129: 1909–1914. https://doi.org/10.1210/endo-129-4-1909 PMID: 1655389

32. Yildiz HM, Carlson TL, Goldstein AM, Carrier RL. Mucus barriers to microparticles and microbes are altered in Hirschsprung’s disease. Macromol Biosci. 2015; 15: 712–718. https://doi.org/10.1002/mabi.201400473 PMID: 25644515

33. Nakamura H, Lim T, Puri P. Inflammatory bowel disease in patients with Hirschsprung’s disease: a systematic review and meta-analysis. Pediatr Surg Int. 2018; 34: 149–154. https://doi.org/10.1007/s00383-017-4182-4 PMID: 28983688

34. Pierce ES. Could Mycobacterium avium subspecies paratuberculosis cause Crohn’s disease, ulcerative colitis… and colorectal cancer? Infect Agent Cancer. 2018; 13: 1. https://doi.org/10.1186/s13027-017-0172-3 PMID: 29308085

35. White SN, Knowles DP. Expanding possibilities for intervention against small ruminant lentiviruses through genetic marker-assisted selective breeding. Viruses. 2013; 5(6): 1466–1499. https://doi.org/10.3390/v5061466 PMID: 23771240