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Correlation between diarrhoea associated SNPs and pre-weaning growth rate in Landlly piglets

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Piglet diarrhoea is an important disease observed in newborn and freshly weaned piglets causing substantial economic losses and serious health risks (Rawat *et al.* 2019). Among bacterial causes, *Escherichia coli* is the most important, responsible for 56.2% of diarrhoeal diseases in piglets and 24.7% of diarrhoea-related mortality globally (Shi 2003). Enterotoxigenic *Escherichia coli* (ETEC) cause diarrhoea and mortality by adhering to receptors on the host's intestinal brush border, particularly in the small intestine. ETEC susceptibility is reported to be genetically controlled resulting from the attachment of *E. coli* fimbria to single or multiple receptors in the piglet's small intestine. Previous linkage studies in pigs have identified a region on SSC13 associated with ETEC susceptibility, with various point mutations impacting adhesion patterns (Sinha *et al.* 2018a, 2018b; Sinha *et al.* 2021). Identifying the mutations responsible for ETEC susceptibility could lead to targeted strategies for managing piglet diarrhoea (Sinha *et al.* 2019) using selection strategy.

Breeding programs incorporating ETEC receptor-negative pigs may help prevent ETEC-induced diarrhoea. A specific genomic region (SSC13q41) harbours polymorphisms in genes such as MUC4, TFRC, MUC13, ACK1, and MUC20, which affect F4 ETEC adhesion to intestinal villi (Sinha *et al.* 2019). Therefore, before performing marker assisted selection, it is essential to investigate the association of such SNPs with other economic traits to avoid co-selection of unwanted traits (Fairbrother *et al.* 2005). Therefore, evaluating the population regarding the impact of selection using diarrhoea associated SNP markers on economic traits is crucial. Additionally, the frequency distribution of SNPs can provide better insight for breeding strategy formulation. Landlly variety of pig is developed at ICAR-IVRI, under the All India Coordinated Research Project (AICRP) on pigs through crossbreeding exotic purebred Landrace and indigenous Ghurrah pig by stabilizing inheritance level at 75% Landrace and 25 % Ghurrah. This crossbred has been successfully disseminated across several states in North and Western India and is known for its outstanding performance in both field and farm conditions. The pre-weaning growth rate is about 176.56 g/day, and post-weaning growth rate is 419.77 g/day, demonstrating consistent and robust growth (Ajay *et al.* 2023).

In order to further strengthen this genetic group in terms of diarrhoea resistance, the scope for selection against diarrhoea is explored. In this context, how selection involving SNPs would affect production traits in Landlly pigs should be essentially known. In piglets growth rate is a crucial factor for determining the marketing weight at weaning time, as it influences both the weaning weight and survival rate of piglets. The present investigation aimed to determine the frequency of diarrhoea-associated polymorphisms in a Landlly population and their association with pre-weaning growth rate which can give some insight for the scope of application of selection for diarrhoea resistance.

Experimental animals and samples/data collection: Blood samples (5 ml) as well as weekly body weight data from 250 Landlly piglets were collected. Genomic DNA was extracted from blood samples using the phenol:chloroform:Isoamyl alcohol method (Sambrook and Russell 2001). DNA quality was assessed with agarose gel electrophoresis and spectrophotometer and only good samples were diluted to 50 ng/µl with nuclease-free water for further use.

PCR-RFLP and mass genotyping: Candidate SNPs were selected based on their reported significant associations with diarrhoea susceptibility in piglets shortlisted from our previous studies from SSC13q41 region (Wagh 2016; Sinha *et al.* 2018a, 2018b; Rawat *et al.* 2019; Sinha *et al.* 2021) and those with significant linkage disequilibrium in

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Ghurrah pigs along with one causative SNP in the FUT1 gene (Meijerink *et al.* 1997). Genotyping of all SNPs were performed using PCR-RFLP method using standard procedure. Information regarding Primer, RE, Expected fragments size on RE digestion and its genotypes for all SNPs of targeted gene and composition of PCR reaction mixture are given in supplementary table 1 and 2. Each PCR product was digested with 1 I.U. of the appropriate restriction enzyme and 10× buffer, then electrophoresed at 120 volts in a 3-3.5% agarose gel (low EEO) for one hour. Band patterns were visualized under UV light and documented. Mass genotyping of all samples was conducted using specific restriction enzymes for each SNP to identify nucleotide sequence variations for 10 SNPs.

Phenotyping for growth rate and statistical analysis: The body weight of all piglets was measured weekly to estimate weekly growth rates. The phenotypic data on growth rate were correlated with the respective genotypic data to assess the impact of ETEC diarrhoea associated SNPs on growth rate. The association between different allelic variants and growth rate was analyzed using a logistic/ categorical model with SAS 9.3 software (SAS Institute Inc., Cary, NC, USA). The PROC ALLELE procedure was used to test Hardy-Weinberg equilibrium (HWE), estimate polymorphism information content (PIC), evaluate SNP marker heterozygosity, and assess linkage disequilibrium (LD) between SNP loci. PROC GLM was utilized to compare body weight gain associated with each genotype.

Gene, genotype frequency and LD of candidate genes: Seven out of 10 SNPs (g.8227 G>C, g.13383 C>T, g.22304 A>G, g.191 C>T, g.107371 A>C, g.93222 C>A, c.307 G>A) were found polymorphic with 2 to 3 genotypes, whereas other three SNPs (g.101030382 T>C, g. 22124 T>C, g.291 C>T) were monomorphic in the target population. The gene and genotypic frequencies of all seven SNPs are presented in Table 1. The CC (367 bp), GC (367 bp, 216 bp, 151 bp) and GG (216 bp, 151 bp) genotypes were observed at g.8227 G>C locus with their frequencies of 48.80% (122), 42.80 % (107), 8.40% (21), respectively. The CC (244) and CT (6) genotypes were observed at g.191 C>T locus with their frequencies of 97.60% (244) and 2.40% (6), respectively. The CC (242) and CA (8) genotypes were observed at *ACK1*-g.93222 C>A locus with their frequencies of 96.80% (242) and 3.20% (8), respectively. The polymorphism information content (PIC) of the studied SNPs ranged from 2.35% (g.22304 A>G) to 37.32% (c.307 G>A) loci in the present population (Table 1). Accordingly, heterozygosity ranged from 2.41% (g.22304 A>G) to 82.73% (c.307 G>A) in the same population. Population statistics of SNPs indicated that the loci g.13383 C>T, g.191 C>T, g.93222 C>A, and c.307 G>A exhibited a significant departure from HWE, unlike all other loci in the present investigation that remained in HWE. The linkage disequilibrium (LD) of

Table 1. Population parameters of all polymorphic SNPs in Landlly population (N= 250)

SNP location	Genotype	Genotypic frequency	Gene frequency	PIC	Heterozygosity	Allelic diversity	Test for HWE Pr>ChiSquare
g.8227 G>C	GG (122)	0.49	G=0.702 C=0.298	0.3314	0.4297	0.4194	0.6966
	GC (107)	0.43					
	CC (21)	0.08					
g.13383 C>T**	CC (202)	0.81	C=0.858 T=0.142	0.2146	0.1004	0.2445	<.0001
	CT (25)	0.10					
	TT (23)	0.09					
g.22304 A>G	AA(127)	0.51	A=0.608 G=0.392	0.0235	0.0241	0.0238	0.8474
	AG (50)	0.20					
	GG (73)	0.29					
g.191 C>T*	CC (244)	0.98	C=0.976 T=0.024	0.3506	0.5261	0.4534	0.0114
	CT (6)	0.02					
g.107371 A>C	AA (98)	0.39	A=0.654 C=0.346	0.0311	0.0321	0.0316	0.7967
	AC (131)	0.52					
	CC (21)	0.08					
g.93222 C>A**	CC (242)	0.97	C=0.968 A=0.032	0.3625	0.2008	0.4756	<.0001
	CA (8)	0.03					
c.307 G>A**	GG (32)	0.13	G=0.542 A=0.458	0.3732	0.8273	0.4964	<.0001
	GA (207)	0.83					
	AA (11)	0.04					

**Indicates significant at P<0.01; *Indicates significant at P<0.05

different loci were tested using the χ^2 probabilities, which revealed that locus g.8227 G>C (*MUC 4*) and g.191 C>T (*MUC 20*), g.13383 C>T (*MUC 4*) and g.191 C>T (*MUC 20*) were significantly (P<0.05) associated with one another.

Association with the growth rate: The association of growth rate (per day and per week) with all polymorphic SNP markers revealed that none of the markers was found to affect the per day growth rate of piglets. However, association of SNPs with per week growth rate revealed that 2 of them have significantly affected the growth rate. The SNPs *MUC 4*-g.8227 G>C was significantly associated with the growth rate at the 2nd to 4th week of age whereas, SNP *MUC 20*-g.191 C>T was significantly associated with the growth rate at 4th week of age (Table 2). Previous reports on the SNP g.8227C>G in exon 7 of the MUC4 gene indicated that allele G was correlated with higher Average Daily Gain (ADG) and Back Fat Thickness (BFT) in Italian Large White pigs, as well as higher ADG in the Italian Landrace breed (Fontanesi *et al.* 2012). Additionally, this SNP was associated with diarrhoea susceptibility in piglets (Fratto *et al.* 2024). The result of the present study supported the findings that SNP *MUC 4*- g.8227C>G had also significantly affected (P<0.05) the growth rate of piglets of the Landlly breed (Table 2). Previous researchers reported that for SNP FUT1-c.307 G>A, animals with the AA genotype showed better average growth and development, possibly due to their resistance to piglet diarrhoea as stated by Wang *et al.* (2012), which we did not observe in this population of Landlly pigs. The antagonistic associations of the *MUC4* g.8227C>G and *MUC20*-g.191 C>T alleles on susceptibility to diarrhoea and growth performances evidence the complexity of applying marker-assisted selection in pig breeding. However, all other SNPs including *FUT1*-c.307 G>A studied under the present investigation was found to act neutral to the preweaning growth rate.

In conclusion, seven out of 10 SNPs were found to be polymorphic. The loci MUC4-g.8227 G>C and MUC20-g.191 C>T were found to be associated with pre-weaning weekly growth rates. These findings indicate that SNPs linked to diarrhoea can also affect the physiological growth rates in pigs, suggesting a complex relationship between growth and disease susceptibility. Notably, the allele associated with higher growth rates is also linked to increased susceptibility to diarrhoea. Therefore, in the breeding program, a balanced approach should be taken to select animals. However, all other SNPs, including FUT1-c.307 G>A, were found to be neutral with respect to the pre-weaning growth rate.

SUMMARY

The present study was aimed to investigate the relationship between diarrhoea-associated polymorphisms and pre-weaning growth rates in an organized Landlly herd. A total of 250 piglets were genotyped for 10 shortlisted SNPs and assessed for pre-weaning growth rates, with body weights recorded from birth to the 6th week of age. Seven out of the 10 SNPs were found to be polymorphic in the target population, with the MUC 4-g.8227 G>C locus significantly affecting growth rates of the 2nd to 4th week piglets whereas, the MUC 20-g.191 affected growth rates at the 4th week of age. These findings suggest that the g.8227C>G and g.191C>T SNP polymorphisms are associated with both growth performance and diarrhoea in piglets, recommending that a balanced approach should be maintained between health status and growth performance

Table 2. Effect of MUC 4-g.8227 G>C and MUC 20-g.191 C>T SNPs on growth rate of pre-weaned Landlly piglets

Age in weeks	SNP MUC 4-g.8227 G>C			SNP MUC 20- g.191 C>T			Overall mean ± SE (kg)
	Genotype	Growth rate±SE (kg)	P Value	Genotypes	Growth rate±SE (kg)	P Value	
1	GG (122)	0.16 ± 0.005	0.98	CC (244)	0.16 ± 0.004	0.33	0.17 ± 0.003
	GC (107)	0.16 ± 0.005		CT (6)	0.19 ± 0.02		
	CC (21)	0.16 ± 0.01					
2	GG (122)^{a}	0.19 ± 0.01	0.03	CC (244)	0.19 ± 0.01	0.20	0.20 ± 0.01
	GC (107)^{a}	0.21 ± 0.009		CT (6)	0.25 ± 0.03		
	CC (21)^{b}	0.15 ± 0.02					
3	GG (122)^{xy}	0.19 ± 0.01	0.03	CC (244)	0.20 ± 0.01	0.18	0.20 ± 0.01
	GC (107)^{x}	0.22 ± 0.01		CT (6)	0.26 ± 0.02		
	CC (21)^{y}	0.16 ± 0.03					
4	GG (122)^{p}	0.20 ± 0.01	0.09	CC (244)^{b}	0.19 ± 0.007	0.01	0.20 ± 0.01
	GC (107)^{p}	0.21 ± 0.01		CT (6)^{a}	0.30 ± 0.01		
	CC (21)^{q}	0.15 ± 0.02					
5	GG (122)	0.21 ± 0.01	0.24	CC (244)	0.21 ± 0.01	0.49	0.21 ± 0.01
	GC (107)	0.21 ± 0.01		CT (6)	0.24 ± 0.01		
	CC (21)	0.17 ± 0.03					
6	GG (122)	0.20 ± 0.01	0.26	CC (244)	0.20 ± 0.01	0.37	0.21 ± 0.01
	GC (107)	0.22 ± 0.01		CT (6)	0.25 ± 0.05		
	CC (21)	0.17 ± 0.03					

Values with same superscript within the columns do not differ significantly.

in piglet selection programs.

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REFERENCES

Ajay A, Chauhan A, Vaishnav S, Rani C, Kumar B, De U K, Verma M R, Singh M and Gaur G K. 2023. Impact of body condition on sow and litter performance, postpartum physiological, hematological, and biochemical parameters in Landlly crossbred pigs. *Tropical Animal Health and Production* **55**(6): 393.

Fairbrother J M, Nadeau É and Gyles C L. 2005. *Escherichia coli* in postweaning diarrhoea in pigs: an update on bacterial types, pathogenesis, and prevention strategies. *Animal Health Research Reviews* **6**: 17-39.

Fontanesi L, Bertolini F, Dall'Olio S, Buttazzoni L, Gallo M and Russo V. 2012. Analysis of association between the MUC4 g. 8227C> G polymorphism and production traits in Italian heavy pigs using a selective genotyping approach. *Animal Biotechnology* **23**: 147-55.

Fratto A, Torricelli M, Sebastiani C, Ciullo M, Felici A and Biagetti M. 2024. Survey on resistance occurrence for F4+ and F18+ enterotoxigenic Escherichia coli (ETEC) among pigs reared in Central Italy regions. *Veterinary Research Communications* **48**(2): 1279-84.

Jacobsen M, Kracht S S, Esteso G, Cirera S, Edfors I, Archibald A L, Bendixen C, Andersson L, Fredholm M and Jørgensen C B. 2010. Refined candidate region specified by haplotype sharing for Escherichia coli F4ab/F4ac susceptibility alleles in pigs. *Animal Genetics* **41**: 21-25.

Ji H, Ren J, Yan X, Huang X, Zhang B, Zhang Z and Huang L. 2011. The porcine MUC20 gene: molecular characterization and its association with susceptibility to enterotoxigenic *Escherichia coli* F4ab/ac. *Molecular Biology Reports* **38**: 1593-601.

Meijerink E, Fries R, Vögeli P, Masabanda J, Wigger G, Stricker C, Neuenschwander S, Bertschinger H U and Stranzinger G. 1997. Two α (1, 2) fucosyltransferase genes on porcine chromosome 6q11 are closely linked to the blood group inhibitor (S) and *Escherichia coli* F18 receptor (ECF18R) loci. *Mammalian Genome* **8**: 736-41.

Rawat C, Sahoo N R, Wagh S S, Kumar P, Kumar S, Sonwane A, Qureshi S, Kumar A and Panigrahi M. 2019. Association of ACK1, TFRC polymorphism with diarrhoeagenic *E. coli* adhesion patterns and their jejunal expression profile in Indian Ghurrah pigs. *Biotech* **9**: 1-10.

Ren J, Tang H, Yan X, Huang X, Zhang B, Ji H, Yang B, Milan D and Huang L. 2009. A pig–human comparative RH map comprising 20 genes on pig chromosome 13q41 that harbours the ETEC F4ac receptor locus. *Journal of Animal Breeding and Genetics* **126**: 30-36.

Sambrook J and Russell D W. 2001. *Molecular Cloning, a Laboratory Manual*, 3rd edition. Cold Spring Harbor Laboratory Press, NY, USA.

Shi Q S. 2003. The review of the receptors of ETEC F4. *Pigs and Poultry* **23:** 33-35.

Sinha R, Sahoo N R, Kumar P, Qureshi S, Kumar A, Ravikumar G V, Bhushan B. 2018(a). Comparative jejunal expression of MUC 13 in Indian native pigs differentially adhesive to diarrhoeagenic *E. coli*. *Journal of Applied Animal Research* **46**: 107-111.

Sinha R, Sahoo N R, Shrivastava K, Kumar P, Qureshi S, Kumar A, Ravi Kumar G V and Bhushan B. 2018(b). Effect of Mucin13 gene polymorphism on diarrhoeagenic *E. coli* adhesion pattern and its expression analysis in native Indian pigs. *Archives Animal Breeding* **61**: 321-28.

Sinha R, Sahoo N R, Shrivastava K, Kumar P, Qureshi S, De U K, Kumar A, Kumar G V and Bhushan B. 2019. Resistance to ETEC F4/F18–mediated piglet diarrhoea: opening the gene black box. *Tropical Animal Health and Production* **51**:1307-20.

Sinha R, Sahoo N R, Shrivastava K, Vineeth M R, Kumar P, Qureshi S, Kumar A and Bhushan B. 2021. Effect of season, age and sex on *E. coli* adhesion patterns in Indigenous Ghurrah pigs-a comparative analysis of phenotypic classifications. *Biological Rhythm Research* **52**: 1217-28.

Van Den Broeck W, Cox E, Oudega B and Goddeeris B M. 2000. The F4 fimbrial antigen of *Escherichia coli* and its receptors. *Veterinary Microbiology* **71:** 223-44.

Wagh S S. 2016. *Genetic polymorphism in porcine mucin 4 and mucin 20 genes and their association with E. coli adhesion pattern*. Master's thesis. IVRI, Izatnagar, U.P, India.

Wang S J, Liu W J, Yang L G, Sargent C A, Liu H B, Wang C, Liu X D, Zhao S H, Affara N A, Liang A X and Zhang S J. 2012. Effects of FUT1 gene mutation on resistance to infectious disease. *Molecular Biology Reports* **39**: 2805–10.