The Pharma Innovation Journal 2022; SP-11(7): 2491-2493

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277-7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2022; SP-11(7): 2491-2493

www.thepharmajournal.com
Received: 02-05-2022
Accepted: 08-06-2022

Soni Kumari
Division of Animal Genetics, ICAR-Indian Veterinary Research Institute, Izatnagar, Uttar Pradesh, India

Bharat Bhushan
Division of Animal Genetics, ICAR-Indian Veterinary Research Institute, Izatnagar, Uttar Pradesh, India

Amit Kumar
Division of Animal Genetics, ICAR-Indian Veterinary Research Institute, Izatnagar, Uttar Pradesh, India

Manjit Panigrahi
Division of Animal Genetics, ICAR-Indian Veterinary Research Institute, Izatnagar, Uttar Pradesh, India

Arnav Mehrotra
Division of Animal Genetics, ICAR-Indian Veterinary Research Institute, Izatnagar, Uttar Pradesh, India

GVPPS Ravi Kumar
Division of Veterinary Biotechnology, ICAR-Indian Veterinary Research Institute, Izatnagar, Uttar Pradesh, India

Anuj Chauhan
Livestock Production and Management Section, ICAR-Indian Veterinary Research Institute, Izatnagar, Uttar Pradesh, India

Waseem Akram Malla
Division of Veterinary Biotechnology, ICAR-Indian Veterinary Research Institute, Izatnagar, Uttar Pradesh, India

Satish Kumar
Division of Animal Genetics, ICAR-Indian Veterinary Research Institute, Izatnagar, Uttar Pradesh, India

GK Gaur
Livestock Production and Management Section, ICAR-Indian Veterinary Research Institute, Izatnagar, Uttar Pradesh, India

Triveni Dutt
Livestock Production and Management Section, ICAR-Indian Veterinary Research Institute, Izatnagar, Uttar Pradesh, India

Corresponding Author
Soni Kumari
Division of Animal Genetics, ICAR-Indian Veterinary Research Institute, Izatnagar, Uttar Pradesh, India

Comparison of gene expression profile in indigenous Ghurrah and exotic Landrace piglets

Soni Kumari, Bharat Bhushan, Amit Kumar, Manjit Panigrahi, Arnav Mehrotra, GVPPS Ravi Kumar, Anuj Chauhan, Waseem Akram Malla, Satish Kumar, GK Gaur and Triveni Dutt

Abstract
Classical swine fever (CSF) is one of the important viral diseases of pigs worldwide. Prophylactic immunization programmes are followed against classical swine fever virus in India. Due to increasing number of antibiotic resistant pathogens and high cost of prophylactic and therapeutic measure, inclusion of health traits in selection program has become important. In this study we have generated the peripheral blood mononuclear cell (PBMC) transcriptomes of indigenous Ghurrah and exotic Landrace pigs breeds. miRNA sequencing data analysis was carried out and 30 DE miRNAs were identified in Ghurrah versus Landrace pre vaccination comparison. *SLC11A1*and *NLRP3* genes were selected for qRT-PCR, which showed concordance in the direction of expression of genes.

Keywords: Ghurrah, landrace, classical swine fever, mirna sequencing

Introduction
Classical swine fever (CSF), formerly known as hog cholera isdevastating viral disease of pigs [1]. It is highly contagious and economically important disease of domestic pigs and wild boars [2]. It has significant impact on pig health due to loss of body weight, loss due to decreased fertility, high mortality and morbidity. The export restrictions on pork and pork products from affected countries cause great loss to the pig industry [3]. CSF is found to be endemic in Asia, Eastern Europe, South and Central America [4]. High Sero-prevalence of CSF (63.3%) in India also suggests that disease is endemic in our country [5]. This disease causes loss of Rs. 4.29 billion annually to the Indian swine farmers [6]. CSF is caused by classical swine fever virus (CSFV) belonging to the genus Pestivirus and family Flaviviridae [1]. CSFV is an enveloped RNA virus having about 12.3 kb genome lengths. CSF Virus has single open reading frame which encodes one polyprotein. This polyprotein is co- and post-translationally processes into four structural and nine non-structural proteins [4]. CSF is highly contagious multi systemic hemorrhagic disease of pigs. It shows acute, subacute, chronic form with late onset and can go unnoticed [1]. CSFV can be transmitted both horizontally and vertically. The most common mean used for prevention and control of CSF in endemic areas is routine vaccination. In India prophylactic vaccination is done by C-strain modified live virus vaccine. This vaccine provides immunity against all CSFV genotypes [7]. miRNAs are ~22 nucleotides small non-coding RNA, which regulate a number of biological processes [8]. After transcription protein coding genes are regulated by miRNAs. miRNAs inhibit translation initiation or elongation of genes. They plays role in degradation of co-translational protein as well as terminates transmission before its completion. miRNAs also regulate genes and biological activities by forming miRNA–lncRNA and miRNA–mRNA pairs [9].

Material and Methods
The study was conducted on randomly selected one Ghurrah and Landrace piglet maintained at All India Coordinated Research Project (AICRP) unit, Indian Veterinary Research Institute (IVRI), Izatnagar. After weaning at 3 months of age, the selected piglets were vaccinated with IVRI strain of lapinized live attenuated CSF vaccine virus. About 10 ml of blood was collected from each piglet prior to vaccination in heparin-coated vacutainers under sterile condition. PBMCs from the whole blood were isolated using density gradient centrifugation using Histopaq 1077 (Sigma Aldrich). PBMCs stored in the RNA later were sent for miRNA sequencing. RNA was extracted from PBMCs using TRIzol reagent (Ambion).

Samples showing RNA integrity number more than 7 were used for library preparation using NEBNext Small RNA Lib prep Kit, following the manufacturer's protocol. The quality of the libraries was checked using Agilent Tapestation. Libraries were sequenced to generate 50 bp single end reads for each sample on Illumina Hiseq 4000 platform. The raw reads were checked using FASTQC (Andrews, 2010). The raw reads were first processed by prinseq-lite to remove low-quality reads. Pig genome was downloaded from Ensembl (https://www.ensembl.org/ downloads.html). The genome was indexed using Bowtie short read aligner program [10]. The deepsequencing reads were processed using miRDeep2 software [11]. The edgeR package was used to identify differentially expressed miRNAs between the two breeds. Target genes of DE miRNAs were predicted using Target scan tool. DE genes obtained from the RNA seq analysis study conducted in our lab were used to identify the common genes between RNA seq data DE genes and predicted genes by Target scan tool. The SLC11A1and NLRP3 gene was selected for validation by qRT-PCR. The primer sequence of these two genes are given in table 1.

Results and Discussion

The present investigation was carried out to identify the differentially expressed miRNAs and validation of DE target genes in Ghurrah and Landrace pigs before CSF virus vaccination. To the best of our knowledge at present very few reports are available regarding the expression profiling of miRNA between the two breeds. The detailed analysis of miRNA seq data by miRDeep2 revealed that 30 miRNAs were significantly differentially expressed, out of which 14 DE miRNAs were upregulated and 16 DE miRNAs were downregulated. Ssc-miR-490-3p was the most upregulated DE miRNA (log2FC = 7.725) while ssc-miR-424-5p was most down regulated DE miRNA (log2FC = -6.985). Upregulated DE miRNAs potentially modulates the expression of *CD28, CD96, CD226, IL18R1, LEF1, KLRK1, SH2D1A, RORA, IFNG, IL2, IL6, and IL12A* genes that are involve in α-β T cell activation, T helper cell differentiation and regulation of natural killer cell mediated immunity. Down regulated miRNAs potentially modulate the expression of *SLC11A1, NFKBIZ, ANXA1, LOXL3, IL1R1, ICAM-1, TNF, NLRP3, CCR1, TLR-2, TLR-8, and TLR-10* genes that are involve in regulation of adaptive immune response, regulation of T helper 1 type immune response, negative regulation of T cell activation. The *SLC11A1and NLRP3*genes were upregulated with log2FC = 2.493 and 2.428, respectively. The fold change obtained by ΔΔCt method of RT- qPCR was compared to the log2 fold change of the gene. Results revealed concordance in the direction of expression of genes (Figure 1). The Solute Carrier family11 member A1 (*SLC11A1*) gene, previously known as Natural Resistance Associated Macrophage Protein 1 (*NRAMP1*) gene is a member of large family of metal ion-transport proteins and it was the first positional cloned gene related to infectious disease susceptibility in mouse [12]. *SLC11A1* gene have pleiotropic effects on macrophage function, that include increased chemokine KC, tumor necrosis factor-α, interleukin-1β, inducible nitric oxidesynthase and major histocompatability complex class II expression; all are important in resistance to intracellular pathogens [13]. A microsatellite polymorphism within the *SLC11A1* gene issignificantly related to lower incidence of bovine tuberculosis in Chadian cattle (African Zebu) [14]. NLRP3, IL-1β and NF-κB were upregulated after Foot and Mouth Disease virus infection. The NLRP3 inflammasome is activated by a wide range of stress signals that are derived from pathogens and environmental stresses. NLRP3 inflammasome can suppress Foot and Mouth Disease virus replication during virus infection [15]. The nucleotide-binding domain and leucine-rich repeat- containing (NLR) family of pattern-recognition molecules mediate host immunity to various pathogenic stimuli.NLRP3 inflammasome as an essential component in host defense against influenza infection through the sensing of viral RNA. Proper activation of an NLR inflammasomeis necessary for host survival and for mediating theinnate immune response after influenza virus infection [16].

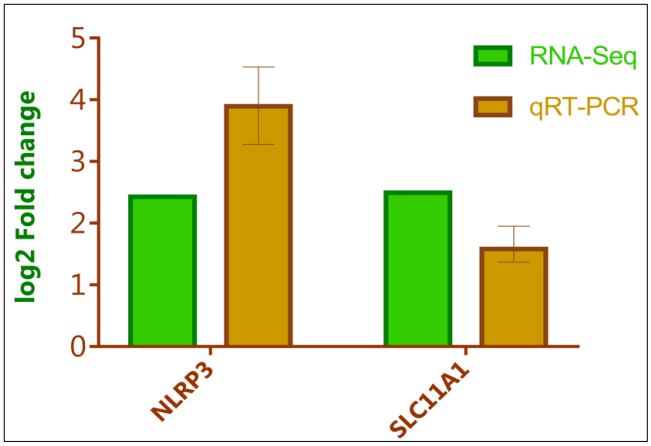

Fig 1: Results revealed concordance in the direction of expression of genes

Table 1: Primers sequence of gene

Gene name	Primer Sequence	Annealing temperature (°C)	Amplicons size
GAPDH	F-ACATGGCCTCCAAGGAGTAAGA	58	106
	R-GATCGAGTTGGGGCTGTGACT		
SLC11A1	F- CTACTGGCTGTCATCTACCT	58	118
	R- CCAGAAGCCCATACAGAAAG		
NLRP3	F- CCAGGGATGAAGGTGTTATG	58	118
	R- TCAGGACAGAGGAGATGTT		

Conflict of interest

The authors declare no conflicts of interest.

Acknowledgement

The authors are thankful to Director, ICAR-IVRI for providing needful facilities. The work was funded by CA Bin project of the ICAR.

References

1. Edwards S, Fukusho A, Lefevre PC, Lipowski A, Pejsak Z, Roehe P, *et al*. Classical swine fever: The global situation. Veterinary microbiology. 2000;73(2-3):103-

119.
2. Chander V, Nandi S, Ravishankar C, Upmanyu V, Verma R. Classical swine fever in pigs: recent developments and future perspectives. Animal health research reviews. 2014;15(1);87-101.
3. Sarma DK, Krishna L, Mishri J. Classical swine fever in pigs and its status in India: A review. The Indian Journal of Animal Sciences, 2008, 78(12).
4. Blome S, Staubach C, Henke J, Carlson J, Beer M. Classical swine fever an updated review. Viruses. 2017;9(4):86.
5. Nandi S, Muthuchelvan D, Ahuja A, Bisht S, Chander V, Pandey AB, Singh RK. Prevalence of Classical Swine Fever Virus in India: A 6-Year Study, 2004-2010. Trans boundary and Emerging Diseases. 2011;58(5):461-463.
6. Singh B, Bardhan D, Verma MR, Prasad S, Sinha DK, Sharma VB. Incidence of classical swine fever (CSF) in pigs in India and its economic valuation with a simple mathematical model. Animal Science Reporter, 2016, 10(7).
7. Van Oirschot JT. Vaccinology of classical swine fever: from lab to field Veterinary microbiology. 2003;96(4):367-384.
8. Do DN, Dudemaine PL, Mathur M, Suravajhala P, Zhao X, Ibeagha-Awemu EM. MiRNA regulatory functions in farm animal diseases, and biomarker potentials for effective therapies. International Journal of Molecular Sciences. 2021;22(6):3080.
9. O'Brien SJ, Carter JV, Burton JF, Oxford BG, Schmidt MN, Hallion JC, *et al*. The role of the miR-200 family in epithelial–mesenchymal transition in colorectal cancer: A systematic review. International journal of cancer. 2018;142(12):2501-2511.
10. Langmead B, Trapnell C, Pop M, Salzberg SL. Ultrafast and memory-efficient alignment of short DNA sequences to the human genome. Genome Biol. 2009;10(3):25-25.
11. Friedlander MR, Mackowiak SD, Li N, Chen W, Rajewsky N. miRDeep2 accurately identifies known and hundreds of novel microRNA genes in seven animal clades. Nucleic Acids Res. 2012;40:37-52.
12. Vidal S, Tremblay ML, Govoni G, Gauthier S, Sebastiani G, Malo D, *et al*. The Ity/Lsh/Bcg locus: natural resistance to infection with intracellular parasites is abrogated by disruption of the Nramp1 gene. The Journal of experimental medicine. 1995;182(3):655-666.
13. Awomoyi AA. The human solute carrier family 11 member 1 protein (SLC11A1): linking infections, autoimmunity and cancer?. FEMS Immunology & Medical Microbiology. 2007;49(3):324-329.
14. Kadarmideen HN, Ali AA, Thomson PC, Müller B, Zinsstag J. Polymorphisms of the SLC11A1 gene and resistance to bovine tuberculosis in African Zebu cattle. Animal genetics. 2011;42(6):656-658.
15. Zhi X, Zhang Y, Sun S, Zhang Z, Dong H, Luo X, *et al*. NLRP3 inflammasome activation by Foot-and-mouth disease virus infection mainly induced by viral RNA and non-structural protein 2B. RNA biology. 2020;17(3):335-349.
16. Allen IC, Scull MA, Moore CB, Holl EK, McElvania-TeKippe E, Taxman DJ, *et al*. The NLRP3 inflammasome mediates in vivo innate immunity to influenza A virus through recognition of viral RNA. Immunity. 2009;30(4):556-565.