The Indian Journal of Veterinary Sciences & Biotechnology ***(2017) Volume 13, Issue 1, 70-76***

ISSN (Print) : 2394-0247 : ISSN (Print and online) : 2395-1176, abbreviated as IJVSBT

***http*://dx.doi.org/10.21887/ijvsbt.v13i01.8738**

Submitted : 27-06-2017 ***Accepted : 30-06-2017*** ***Published : 16-08-2017***

Validation of Interferon Induced IFIT Genes in Host Antiviral Defence Against BTV16 Infection : An Initial Report

Manimegalai J, Minakshi Prasad*, Basanti Brar, Ikbal, Ravi Kumar Gandham, Upendra P Lambe, Koushlesh Ranjan and Jyoti Misri

Department of Animal Biotechnology, LLR university of Veterinary and Animal Sciences, Hisar, Haryana, India, 125004

Corresponding Author: minakshi.abt@gmail.com

This work is licensed under the Creative Commons Attribution International License (http://creativecommons.org/licenses /by/4.0/P), which permits unrestricted use, distribution and reproduction in any medium, provided the original work is properly cited.

Copyright @: 2016 by authors and SVSBT.

Abstract

Whole blood samples were collected from healthy sheep and goat, Peripheral blood mononuclear cells (PBMC) isolation was done from whole blood of both the species and cultured in six well plates using RPMI-1640 media in the presence of 5% CO_2 at 37 ^{0}C. Bluetongue (virus BTV)-16 infection was given in PBMCs of both the species at 0 h and representative samples were collected at 24, 48 and 72 h. Total RNA was extracted and cDNA was obtained by reverse transcription. All the four differentially expressed genes (IFITs genes) were validated using quantitative real time PCR and the target gene expression was studied in fold change at 24, 48 and 72 h post infection of sheep and goat PBMCs against BTV-16 infection along with mock infected cells as control. Up regulation in target gene expression was found in virus infected PBMCs of both the species at all the time intervals than control PBMCs indicates the antiviral host protective response of those DEGs against BTV-16 infection. NS1 gene expression of BTV16 in sheep and goat PBMCs at different time interval were also determined which showed significant variation in both species.

Introduction

BT is an economically important multispecies disease which causes severe economic losses in terms of morbidity, mortality, animal trade across the boundaries and various production and reproduction losses (Osburn et al., 1981). After entry of BT virus in host body, virus replicates in monocytes, lymphocytes, dentritic cells along with several other cell types such as lymphoid tissues, lungs and skin which can be easily visualized through confocal microscopy (Singh et al., 2017). Generally peripheral blood mononuclear cells (PBMCs) are used for *in-vitro* culture to study the innate immune responses in sheep and goat against BTV infection (Dhanasekaran et al., 2013).

Recently global transcriptome based analysis was used to understand the molecular events of host virus interaction in PPRV (Manjunath et al., 2015), measles virus (Bolt et al, 2002; Nanda et al., 2009), rinderpest virus (Nanda et al., 2009) and bluetongue virus (Singh et al., 2017). Transcriptome analysis of sheep and goats PBMCs infected with BTV-16 has shown the differentially up regulation of immune system activator and Interferon stimulator genes such as IFIT1 (ISG56), IFIT2 (ISG54), IFIT3 (ISG60) and STAT2 which are considered as responsible for antiviral activity in the host (Singh et al., 2017). STAT2 transcription factor is commonly identified in co-regulation of (differentially

expressed genes) DEGs with its network genes which are down regulated in sheep but up regulated in goats (Singh et al., 2017). Secretion of interferons (IFNs) from virus-infected cells is a hallmark of host antiviral immunity. Most mammals possess IFIT1, IFIT2, IFIT3 and IFIT5 proteins which are involved in innate immune response regulation. They are considered as important targets with potent antiviral activities (Zhou et al., 2013). In the present study, the role of differentially expressed IFIT gene was identified in PBMCs of healthy sheep and goat against BTV-16 infection at 24, 48 and 72 h time intervals and validated for its host antiviral response using Real-time PCR.

Materials and methods

Blood collection

Whole blood was withdrawn by jugular puncture from healthy sheep and goat in heparinized vacutainers using aseptic precautions. Freshly collected blood was allowed to stand at room temperature for 30 minutes before further processing.

Isolation of PBMC from whole blood

Heparinized whole blood was layered over histopaque-1077 medium and centrifuged at 2000 rpm for 30 minutes. Interphase layer rich in PBMC was transferred into a fresh sterile tube and three times washings were given in RPMI-1640 media by gentle mixing and spinning at 1800 rpm for 10 minutes each. Final pellet was re-suspended in RPMI-1640 media with 10 percent fetal calf serum. The PBMCs cells from sheep and goat were seeded in six well plates at the rate of 2 x 10^6 cells per ml and incubated at 37°C in presence of 5% CO_2 (Singh et al, 2017).

Infection of PBMC with BTV-16

PBMCs from sheep and goat were seeded in six well plates separately and infection was given using BTV-16 at 10^5 $TCID_{50}$ /ml along with mock infected cells which serves as control. Infected PBMCs were observed for cytopathic changes such as syntitial formation, viral inclusion bodies and virus specific tubule formation at regular interval of 24, 48 and 72 h post infection. Representative samples were collected from infected and mock infected cells at all the time intervals.

RNA isolation and cDNA synthesis

RNA was extracted from PBMCs of infected and control samples taken at different time intervals by TRIZOL method. The RNA sample extracted was quantified using nanodrop. The double stranded cDNA was synthesized using revert aid first strand cDNA synthesis Kit (thermo scientific) using random hexamer primer. Since the NS1 gene of BTV is conserved in all the serotypes, NS1 gene based PCR and Real time PCR was done to confirm the BTV infection and replication in PBMCs at different time intervals.

Quantitative real time PCR

The real time PCR reaction was carried out in a 20 µl reaction volume, containing 10 µl of Eva green Master mix (G Biosciences, USA), 1µl of forward and reverse primer (final concentration 5 pmol each), 1 µl of diluted cDNA sample (equivalent to 5 ng of RNA), 8 µl of nuclease-free water. The PCR cycling and thermal conditions were kept as initial denaturation at 95°C for 10 min followed by 40 cycles of 95°C for 15 sec, 60°C for 30 sec, and 72°C for 45 sec. The specificity of the product was confirmed by melt curve analysis. The IFIT genes (IFIT1, IFIT2 and IFIT3) expressions were validated using glyceraldehyde 3-phosphate dehydrogenase (GAPDH) as an endogenous control. The relative gene expression analysis of these target genes IFIT (IFIT1, IFIT2 and IFIT3) were carried out using MS excel package. NS1 gene expression was studied with thermal conditions, initial denaturation at 95°C for 10 min followed by 40 cycles of 95°C for 15 sec, 54°C for 30 sec, and 72°C for 30 sec and specificity was confirmed by melt curve. Gene expression was determined by using the values of standard curve.

Results and discussion

Both infected and mock infected PBMCs were observed for any cytopathic changes during 24, 48 and 72 h post-infection (p.i). At 24 h p.i. no significant cytopathic changes except aggregation of few cells were observed on microscopic examination of PBMCs of both species. At 48 h p.i aggregation of cells started and only few dead cells were noticed in both the species. However, sheep PBMCs exhibited changes such as cellular death when compared to goat PBMCs. At 72 h p.i syncytial formation and cell death was prominently observed in sheep PBMCs which indicates that sheep PBMCs are more susceptible to BTV in comparison to goat PBMCs (Figure 1 and 2). Mock infected PBMCs of both species were healthy and no changes were observed at time intervals of 24, 48 and 72 h respectively. The ns1 gene expression is the hallmark of viral replication in all BTV serotypes (Maan et al., 2011). Presence of NS1 gene amplicon of 274 bp in PCR amplification showed the replication of BTV-16 in PBMCs of both sheep and goat species at time intervals 24, 48 and 72 h post infection (p.i.). The significant increase in expression of NS1 gene was observed from 24 h p.i. to 72 h p.i in both sheep and goat PBMCs indicating the increase in replication of virus with time interval (Figure 3). The cDNA from the mock-infected cells showed no amplification of the NS1 gene.

The expression of four differentially expressed genes (IFITs) in PBMCs against BTV-16 infection was validated by Quantitative Real time PCR using GAPDH as an endogenous control for normalization of target gene of interest. The relative gene expression of each target gene for both the species (sheep and goat) was validated with GAPDH as endogenous control using $2^{-\Delta\Delta CT}$ method with control group as calibrator (Schmittgen, 2008). Target Gene expression is high in virus infected samples of both the species in comparison to mock infected samples which showed the antiviral response of those differentially expressed genes against BTV-16 infection. The

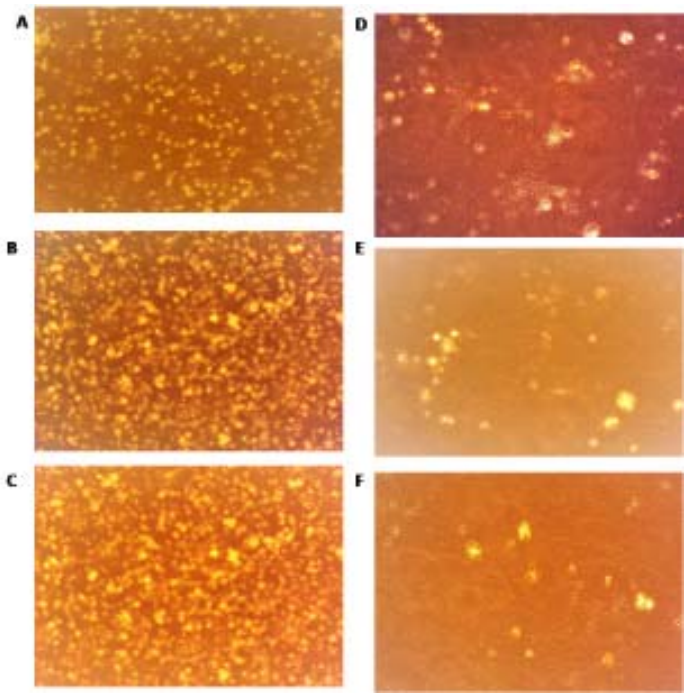

Figure 1: Sheep PBMCs infected with BTV-16 and mock infected Sheep PBMCs. A- Control 24 hour, B- Control 48 hour, C- Control 72 hour, D- Post infection 24 hour, E- Post infection 48 hour, F- Post infection 72 hour

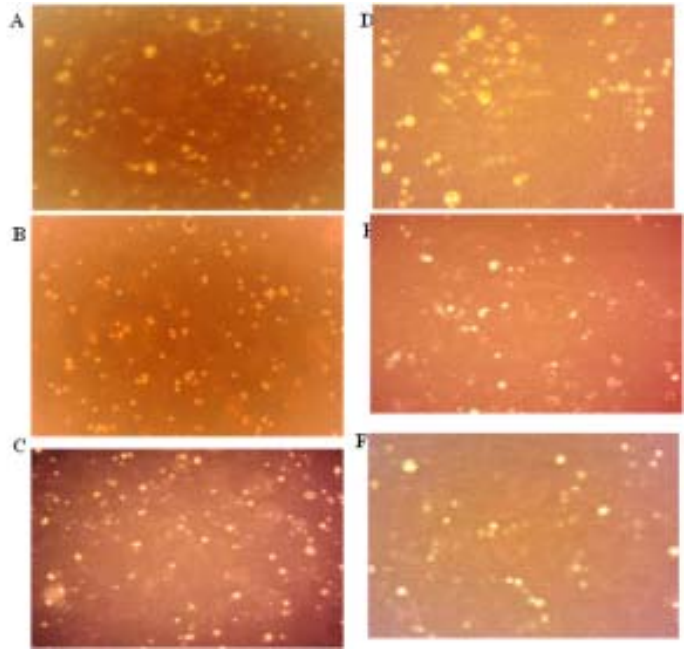

Figure 2: Goat PBMCs infected with BTV-16 and mock infected Goat PBMCs; A- Control 24 hours, B-Control 48 hours, C- Control 72 hours, D- Post infection 24 hours, E- Post infection, 48 hours, F- Post infection 72 hours.

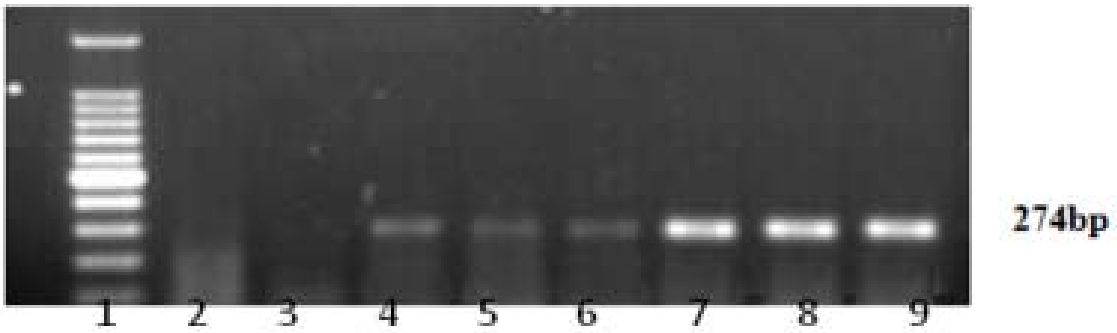

Figure 3: PCR amplification of NS1 gene of BTV-16 in PBMCs of Sheep and Goat at 24h, 48h and 72 h Post infection (p.i.). Lanes 1- Ladder 100bp, 2- Sheep control, 3- Goat control, 4- Sheep 24 Hour p.i, 5- Goat 24 Hour p.i, 6- Sheep 48 Hour p.i, 7- Goat 48 Hour p.i, 8- Sheep 72 Hour p.i, 9- Goat 72 Hour p.i

changes in target gene expressions were calculated using MS Excel package for both the species along with the standard error of difference (Figures 4 and 5). A melt curve analysis was performed

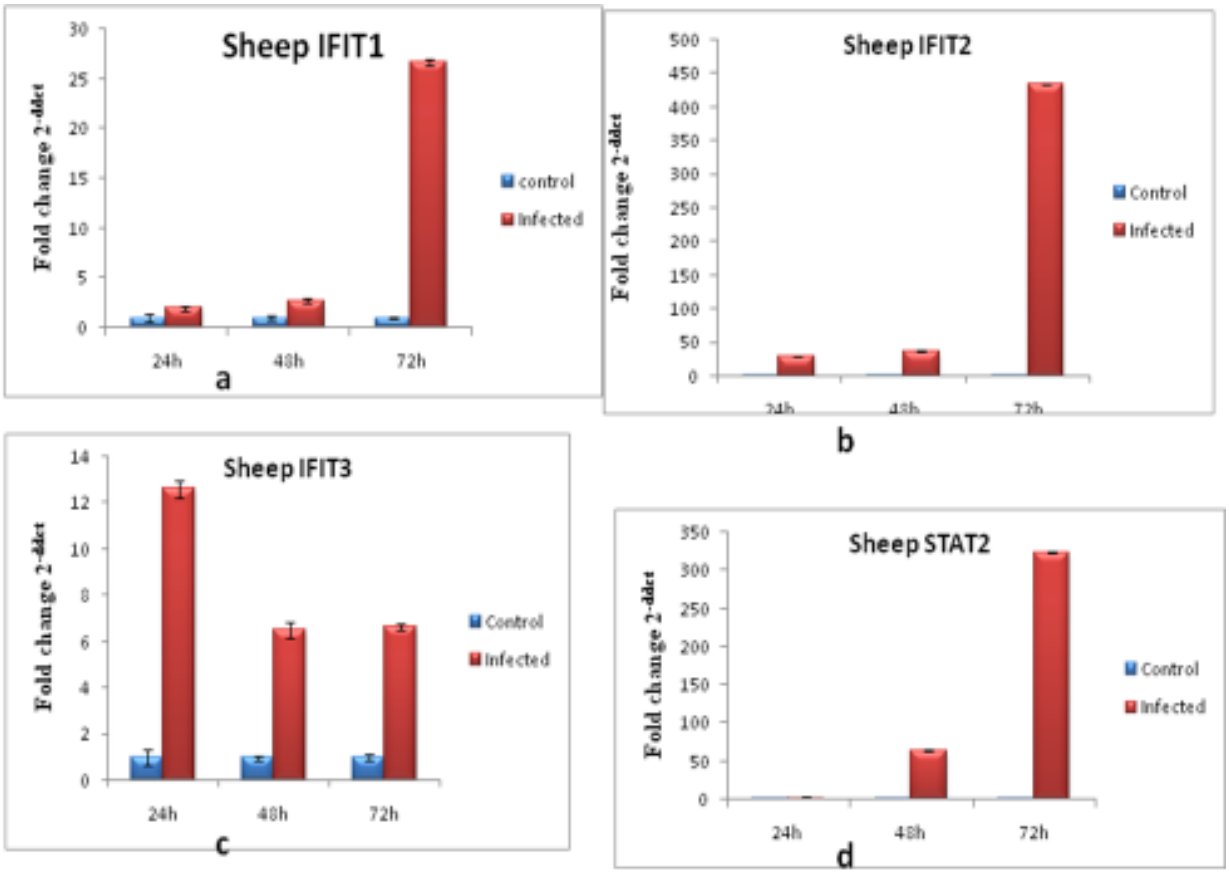

Figure 4: Fold change genes expression in sheep PBMC; a) Fold change in IFIT1 gene expression in sheep PBMC b) Fold change in IFIT2 gene expression in sheep PBMC, c) Fold change in IFIT3 gene expression in sheep PBMC, d) Fold change in STAT2 gene expression in sheep PBMC.

to know the specificity of the qPCR. Target gene expression in respect to fold change ($2^{-\Delta\Delta CT}$) was higher in BTV-16 infected PBMCs of sheep than goat PBMCs and mock infected PBMCs. The NS1 gene expression was also studied in both the species at different time interval by qPCR to compare the NS1 gene expression between time intervals within species. The relative gene expression of NS1 gene (Figure 6) and other DEGs was found comparatively higher in sheep PBMCs. It may

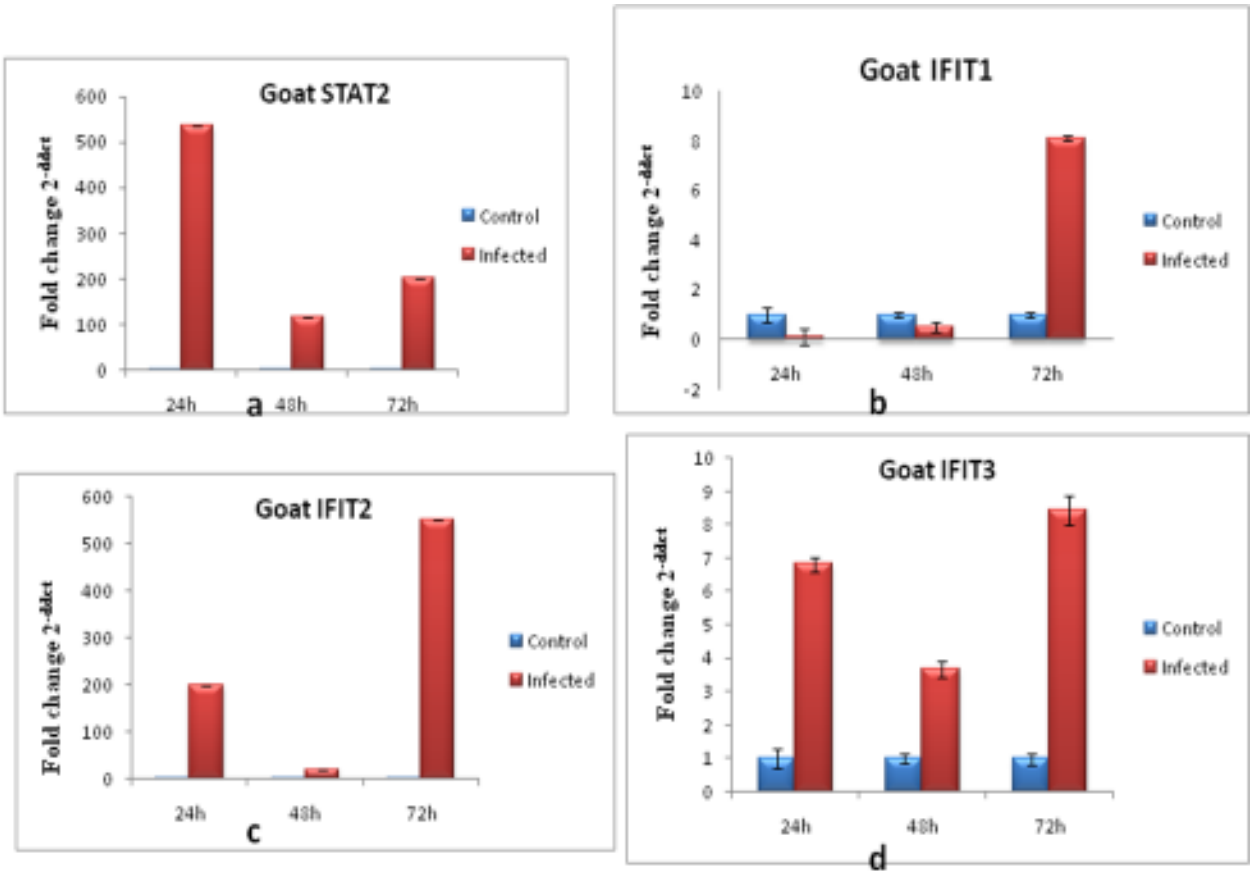

Figure 5: Fold change genes expression in goat PBMC; a) Fold change in STAT2 gene expression in goat PBMC b) Fold change in IFIT1 gene expression in goat PBMC, c) Fold change in IFIT2 gene expression in goat PBMC, d) Fold change in IFIT3 gene expression in goat PBMC.

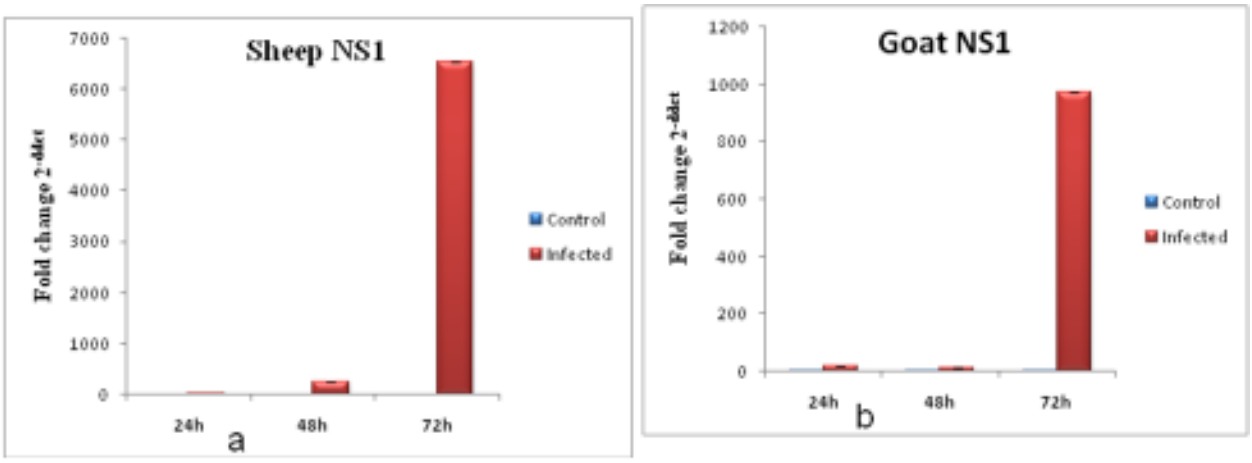

Figure 6: Fold change of NS1 gene expression a) Sheep PBMC, Goat PBMC

be due to the fact that sheep PBMCs support higher virus replication than goat PBMCs (Dhanasekaren et al., 2013; Singh et al., 2017). Vishwaradhya et al. (2013) reported the sensitive detection of novel Indian isolate of BTV 21 using NS1 gene based real-time PCR assay. As per the RNA-seq data of global transcriptome analysis, positive shift in immune response to BTV-16 was found higher in goat than sheep at 72 h post infection So the up regulation of all the four genes IFIT1, IFIT2, IFIT3 and STAT2 at 24 h, 48 h, and 72 h of post infection indicates that the positive

shift in immune response and maintenance in anti-viral state in the host with respect to RNA-seq data (Singh et al., 2017). Terenzi et al. (2008) and Saikia et al. (2010), reported that IFIT1 can inhibit infection with human papillomavirus (HPV), by binding the viral E1 helicase which is required for replication of virus. Most of the IFIT family gene's promoter region has two exons and two or three IFN-stimulated response elements (IRSE) (Kusari and Sen, 1981). These IRSE are the important cis-acting elements recognized by IFN- stimulated gene factor-3 (Fenster and Sen, 2011). Understanding the variants in IFIT genes help in elucidating the immune mechanism and thus influence therapy of infectious diseases in humans and animals. Studying the structural divergence and expression diversities of these genes helps in inventing effective vaccine strategies to combat viral outbreaks.

Conclusion

Expression of this IFIT family genes are considered as hallmark in case of viral infection which can induce the host antiviral immune response that protects the host from major devastation. Further studies need to be conducted to elucidate the immune mechanism for the discovery of antiviral drugs and vaccines.

Conflict interest: All authors declare that they have no conflict of interest.

Acknowledgements

Authors are thankful to ICAR, New Delhi for providing financial support under 'All India network programme on Bluetongue' and Department of Animal Biotechnology, LLR University of Veterinary and Animal Sciences, Hisar, for providing infrastructural facility.

References:

Bolt, G.; Berg, K.; Blixenkrone-Moller.M. (2002). Measles virus induced modulation of host-cell gene expression. Journal of General Virology **83**, 1157–1165.

Dhanasekaran.S.;Vignesh, A.R.; Raj, G.D.; Reddy, Y.K.; Raja, A.; Tirumurugaan, K.G. (2013). Comparative analysis of innate immune response following in vitro stimulation of sheep and goat peripheral blood mononuclear cells with bluetongue virus - serotype 23. Vet. Res. Commun. **37**: 319–327.

Fenster, V.; Sen, G.C. (2011). The ISG56/IFIT1 gene family. J Interferon Cytokine Res. **31**:71–78.

Kusari, J.; Sen, G.C. (1987). Transcriptional analyses of interferon-inducible mRNAs. Mol Cell Biol. **7**: 528–531.

Maan, S., Maan, N.S., Nomikou, K., Veronesi, E., Bachanek-Bankowska, K., Belaganahalli, M.N., Attoui, H., Mertens, P.P. (2011). Complete genome characterization of a novel 26th bluetongue virus serotype from Kuwait. PLoS One. **6**(10): e26147.

Manjunath, S.; Kumar, G.R.; Mishra, B.P.; Mishra, B.; Sahoo, A.P.; Joshi, C.G.; Tiwari, A.K.; Rajak, K.; Janga, S.C. (2015). Genomic analysis of host – Peste des petits ruminants vaccine viral transcriptome uncovers transcription factors modulating immune regulatory pathways. *Vet. Res.***46**: 15.

Nanda, S.K.; Baron, J.; Royall, E.; Robinson, L.; Falciani, F.; Baron, M.D. (2009). Infection of bovine dendritic cells by rinderpest or measles viruses induces different changes in host transcription. Virol. **395**: 223–231.

Osburn B.I.; McGowan, B.; Heron, B.; Loomis, E.; Bushnell, R.; Stott, J.; Utterback, W. (1981). Epizootiologic study of bluetongue: virologic and serologic results. Am. J. Vet. Res.**42**: 884–887.

Saikia P., Fensterl V, Sen GC (2010). The inhibitory action of P56 on select functions of e1 mediates interferon's effect on human papillomavirus DNA replication. J. Virol. **84**:13036–13039.

Schmittgen, T.D. and Livak, K.J. (2008). Analyzing real-time PCR data by the comparative C (T) method. Nat. Protoc. **3**: 1101–1108.

Singh, A.; Prasad, M.; Mishra, B.; Manjunath, S.; Sahu, A.R.; Priya, G.B.; Wani, S.A.; Sahoo, A.P.; Kumar, A.; Balodi, S.; Doera, A.; Saxena, S.; Gandham, R.K. (2017). Transcriptome analysis reveals common differential and global gene expression profiles in bluetongue virus serotype 16 (BTV-16) infected peripheral blood mononuclear cells (PBMCs) in sheep and goats. Genom Data.**11**: 62-72.

Terenzi F, Saikia P, Sen GC (2008). Interferon-inducible protein, P56, inhibits HPV DNA replication by binding to the viral protein E1. Embo. J. **27**:3311–3321.

Vishwaradhya T.M., Minakshi P., Ranjan K., Supriya, Kumar P., Prasad G. 2013. Vet world. **6**(8): 554-557.

Zhou, X.; Michal, J.J.; Zhang, L.; Ding, B.; Lunney, J.K.; Liu, B.; Jiang, Z. (2013). Interferon induced IFIT family genes in host antiviral defense. Int. J. Biol. Sci. **9**(2): 200-208.

❑