

## Analysis of Codon Contribution Between the *Pestivirus* Genus and Their Natural Hosts

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**Abstract:** Pestiviruses including Classical Swine Fever Virus (CSFV), Bovine Viral Diarrhea Virus (BVDV) and Border Disease Virus (BDV) can cause serious livestock diseases. The genetic characteristics between the three viruses and their hosts were analyzed by the Relative Synonymous Codon Usage value (RSCU) based on principle component analysis method. To compare with the overall codon usage between the three pestiviruses and their hosts, the overall codon usage of CSFV is away from BVDV and BDV and some strains of CSFV are strongly separated from others strains. These results indicate that although a strong mutation pressure from the three RNA viruses takes part in these viruses' evolution by the alternation of synonymous codons, translation selection from their hosts on the synonymous codon usage pattern of the viral whole coding sequences should not be ignored. Moreover, the codon distribution of the three pestiviruses relatively adapts to the overall codon usage pattern of their hosts. The general codon usage patterns of the three pestiviruses are the equilibrium of mutation pressure from virus and translation selection from host.

**Key words:** Pestivirus, genetic characteristic, the overall codon usage, mutation pressure, translation selection, hosts

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### INTRODUCTION

The degeneracy of the genetic code causes synonymous codons to encode the same amino acid. However, synonymous codons are not used randomly, namely codon usage bias. The evolutionary phenomenon can be caused by the two main different factors of mutational pressure and translational selection (Cutter *et al.*, 2006; Duret and Hurst, 2001; Plotkin *et al.*, 2004). In term of human RNA viruses, it has been observed that codon usage bias is caused by mutational pressure, G + C context, the segmented property of the genome and the route of transmission of the virus (Drake, 1993; Karlin *et al.*, 1994). Although, it is known that mutation pressure and translation selection are the more generally accepted mechanisms accounting for codon bias (Coleman *et al.*, 2008; Karlin *et al.*, 1990; Liu *et al.*, 2010; Zhi *et al.*, 2010; Zhou *et al.*, 1999, 2010) other selection forces have also been proposed such as fine-tuning translation kinetics selection as well as escape of cellular antiviral responses (Aragones *et al.*, 2008, 2010; Sugiyama *et al.*, 2005). Comparison of the overall codon

usage pattern can play an important role in the understanding of viral evolution, particularly the comparison of codon usage pattern between RNA viruses and their hosts (Bahir *et al.*, 2009; Liu *et al.*, 2011; Sanchez *et al.*, 2003). Clearly, comparison of the overall codon usage patterns between RNA viruses and their natural hosts can reveal much about the molecular evolution of RNA viruses. Such information would be relevant in understanding the regulation of viral gene expression based on viral codon preference.

Pestiviruses which belong to the Flaviridae family have positive-stranded, nonpolyadenylated RNA genomes which encodes one large Open Reading Frame (ORF) flanked by 5' and 3' Nontranslated Regions (NTR). This genome codes for 4 structural proteins, the Capsid<sup>®</sup> and 3 Envelop proteins (E<sup>ms</sup>, E1 and E2), plus 7 Non-Structural proteins (p7, NS2, NS3, NS4A, NS4B, NS5A and NS5B). The pestiviruses can lead to serious livestock pathogens which mainly own Bovine Viral Diarrhea Virus (BVDV) of cattle, Classical Swine Fever Virus (CSFV) of swine and Border Disease Virus (BDV) of sheep. For the range of susceptible animals of the three viruses, CSFV

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has a restricted natural host range which only contains domestic pigs and wild boars (Carbrey *et al.*, 1976; Laddomada, 2000) BVDV owns a wide range of natural hosts for example, buffalo, roe deer, red deer, wild boar, bigborn sheep and causes many respiratory and reproductive problems for example, diarrhea, mucosal disease or abortion in sheep and cattle (Vilcek and Nettleton, 2006), BDV infects sheep causing principally reproductive disease or abortion in cattle and sheep, this virus was sporadically found in pigs as well (Becher *et al.*, 1997; Nettleton *et al.*, 1998; Roehe *et al.*, 1992).

Adaptation and co-evolution of RNA viruses to susceptible animals were mostly studied by analyzing alternation at non-synonymous and synonymous coding sites in specific genes for example the adaptation of human immunodeficiency virus-1 to specific HLA-1 epitopes (Kawashima *et al.*, 2009). The study of adaptation of viruses toward their hosts has been also undertaken for specific viral families (Wong *et al.*, 2010). There is no doubt that accumulating data on the genetic characteristics of CSFV, BVDV, BDV and the relationship of codon usage pattern between them and their natural susceptible animals will contribute to a better understanding of pestivirus evolution and significantly improve molecular epidemiology of pestivirus infections as well. Despite the importance of the codon usage pattern as an indicator of the forces shaping genome evolution, a systemic analysis is not applied for the extent and origin of the codon bias in pestiviruses. To investigate the potential effects of the codon usage pattern of the susceptible animals on the codon preference in these viruses. The researchers analyzed the relationship of the codon usage pattern between the three pestiviruses and three susceptible livestock, particularly the contributions made by translation selection from hosts and mutation pressure by the viruses themselves.

## MATERIALS AND METHODS

**Data of virus sequences and codon usage in hosts:** Based on the earlier report about information of the whole coding sequences of BDV, CSFV and BVDV (Zhou *et al.*, 2012), the Open Reading Frame (ORF) of the three viruses were obtained from the National Center for Biotechnology Information (NCBI) (<http://www.ncbi.nlm.nih.gov/Genbank/>). Moreover, to estimate the link between the natural hosts and the three viruses in the synonymous codon usage pattern, synonymous codon usage frequencies of sheep (*Ovis aries*), cattle (*Bos taurus*) and pig (*Sus scrofa*) were gotten from the codon usage database (Nakamura *et al.*, 2000).

### **The calculation of the Relative Synonymous Codon Usage (RSCU):**

To identify the synonymous codon usage patterns eliminating the confounding affection of amino acid composition among different genes, the Relative Synonymous Codon Usage (RSCU) values for synonymous codons of the three viruses were calculated followed by the formula provided from the earlier report Sharp *et al.*, 1986). Stop codons (UAA, UAG and UGA), AUG for Met and UGG for Try are excluded from the RSCU calculation. For each ORF of the three pestiviruses and the synonymous codon usage frequencies of sheep, cattle and pig, the RSCU data of the 59 synonymous codons were calculated. Additionally, the comparative analysis of the general codon usage pattern between the ORFs of the three pestiviruses and the natural hosts was performed to estimate the adaptation of the pestiviruses to the corresponding the susceptible host.

### **Principle component analysis:**

To estimate the discrepancy between the overall codon usage pattern of each pestivirus and that of natural host, Principal Component Analysis (PCA) was employed. PCA is a useful method reducing the data dimensionality by performing a covariance analysis between factors. The method can provide a direct way to visualize the link in the overall codon usage pattern between the codon distribution of the viruses and their natural susceptible host (domestic pig, cattle and sheep). Researchers can establish each two-dimensional map which provides the evolution distance between the virus and its host at the aspect of codon usage and a relationship between the codon distribution of the virus and the overall codon usage pattern of the host. The two analysis methods were carried out by the Statistical Software SPSS 11.5 for windows. Graphs were plotted using Sigmaplot 10.0 (Systat Software Inc.).

## RESULTS AND DISCUSSION

### **The genetic characteristic of the three viruses at the aspect of codon usage:**

Projection of the overall codon usage by ORFs, onto the two-dimensional map derived from PCA showed that the genetic diversity of the three viruses. In general by comparing between the overall codon usage of the three viruses and those of cattle, sheep and pig, researchers found that a large evolution distance existed between the RNA viruses and hosts (Fig. 1) suggesting that RSCU data can be applied to estimate the genetic diversity. In term of the genetic diversity of the three pestiviruses, the overall codon usage of CSFV was away from BVDV and BDV and some

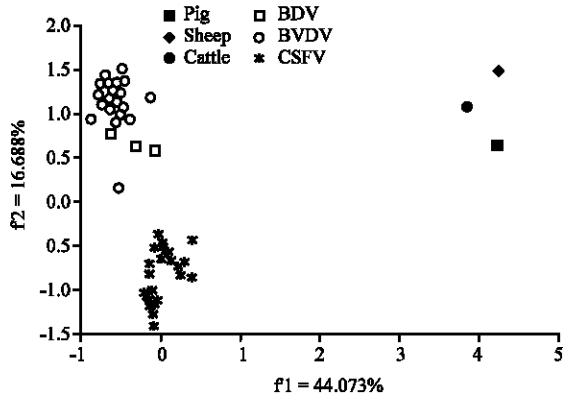


Fig. 1: The discrepancy between the overall codon usage pattern of the three pestiviruses and those of cattle, sheep and pig. The two-dimensional map was established by the first principle component values (f1 data) and the second principle component values (f2 data) derived from the 59 synonymous codon usage patterns in the three pestiviruses and the three hosts. The f1 data accounts for 44.073% and the f2 data accounts for 16.688% of total vibrations

strains of the three viruses were strongly separated from others of CSFV (Fig. 1). Compared with CSFV which generally infected the pig family, the overall codon usage pattern of BVDV was similar with that of BDV (Fig. 1). It is interesting that although the second principle component values (f2 data) approximately ranged from -1.5 to 1.5, the first principle component values (f1 data) approximately ranged from -1 to 0.5 suggesting that despite of high mutation rate, the overall codon usage of the three viruses were limited by some selection pressure.

**Visualizing codon usage patterns between CSFV, BVDV, BDV and cattle, sheep and pig:** Figure 2-4 also made immediately obvious the relationship between the overall codon usage patterns of the three livestock and the codon distribution of CSFV, BVDV or BDV, respectively. Generally, they confirmed that the cells deriving from the three livestock were the candidate systems for expressing proteins encoded with CSFV, BVDV and BDV codon usage profiles. The three maps also suggested that the three viruses might express in the three livestock cells successfully in theory. Furthermore, the relationship assists to visualize the synonymous codons which are chosen differentially by each of the three livestock. Additionally, it was noted that the usage of AGA, AGG, UCG and ACU of CSFV, the usage of AGG, AGA of BVDV and the usage of AGA, AGG, CCA and UCA of BDV were obviously apart from the usages of the other codons

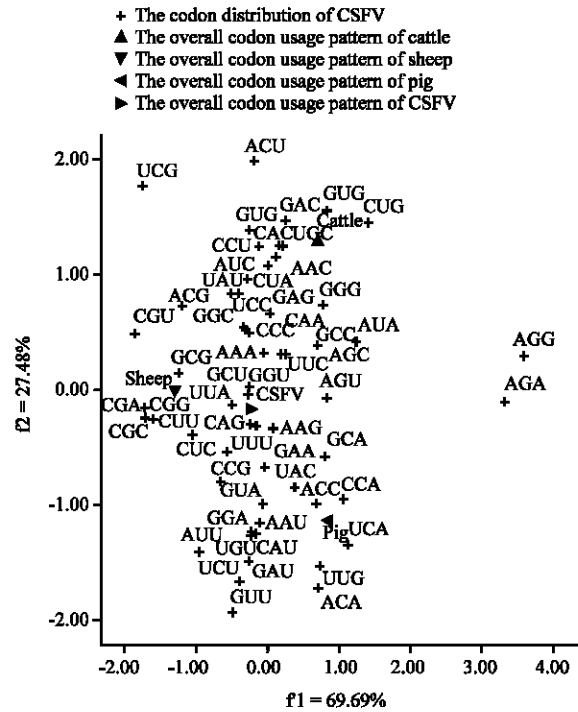


Fig. 2: Graphical representation of the relationship between the codon distribution of CSFV and the overall codon usage pattern of the three livestock. The two codons ATG and TGG that uniquely encode Met and Trp, respectively have been omitted. Two dimensions were identified that accounted for 69.69% (f1 data) and 27.48% (f2 data) of the total codon variability information, respectively

(Fig. 2-4). These results might indicate that the synonymous codon usage patterns of these codons mentioned earlier might be used as a genetic mark for the corresponding virus.

The overall codon usage pattern can reflect a genetic diversity of viruses and the reaction between some RNA viruses and their hosts has been noted (Liu *et al.*, 2011; Sanchez *et al.*, 2003). This study of the overall codon usage pattern between the three pestiviruses and their hosts showed genetic and ecological characteristics. Comparative genomics analysis has been focused on the ongoing evolution of the codon usage pattern of different organisms (Gustafsson *et al.*, 2004; Knight *et al.*, 2001; Santos *et al.*, 2004), the different synonymous codon usage patterns in different organisms is a barrier to heterologous expression. Since, the viruses depend on their host cell's machinery for its replication, codon usage bias could play an important role in host adaptation and the virulence of the viruses. Compared with the range of

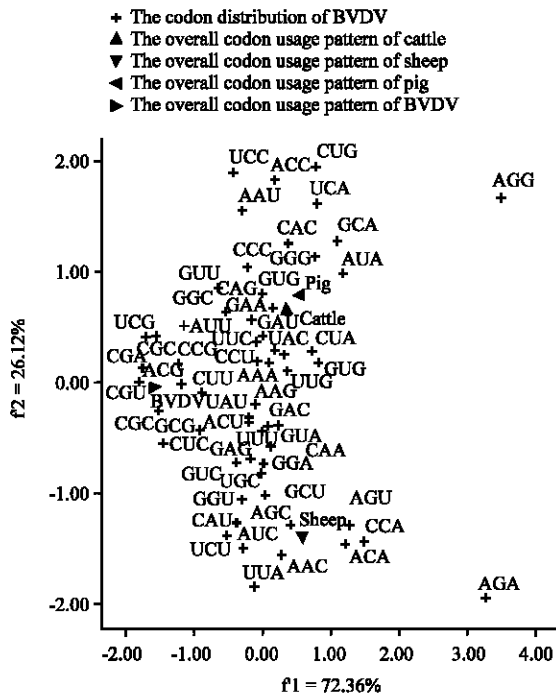


Fig. 3: Graphical representation of the relationship between the codon distribution of BVDV and the overall codon usage pattern of the three livestock. The two codons ATG and TGG that uniquely encode Met and Trp, respectively have been omitted. Two dimensions were identified that accounted for 72.36% (f1 data) and 26.12% (f2 data) of the total codon variability information, respectively

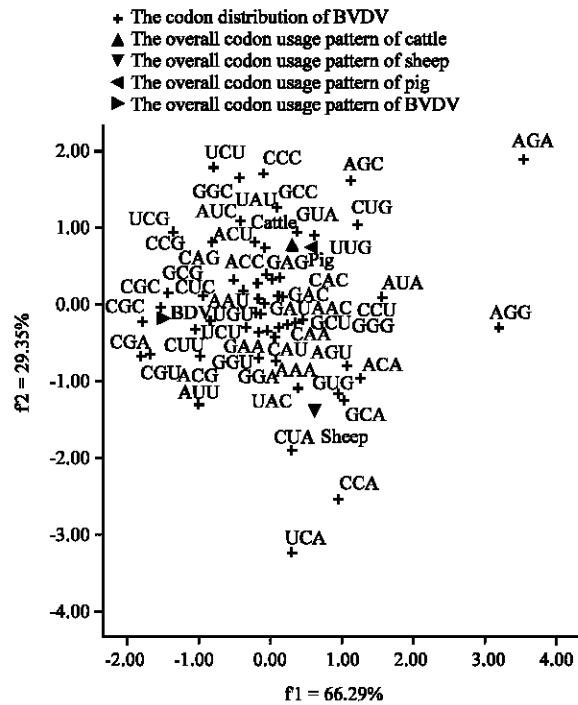


Fig. 4: Graphical representation of the relationship between the codon distribution of BDV and the overall codon usage pattern of the three livestock. The two codons ATG and TGG that uniquely encode Met and Trp, respectively have been omitted. Two dimensions were identified that accounted for 66.29% (f1 data) and 29.35% (f2 data) of the total codon variability information, respectively

susceptible animals of BVDV and BDV, CSFV infecting only wild boars and domestic pig is an interesting genetic phenomenon. From Fig. 1, the distinct evolutionary feature of the overall codon usage pattern of the three viruses indicated that the different species of host can influence the genetic characteristics of the corresponding virus.

This genetic characteristic may be supported by adaptation of CSFV to its natural host (domestic pigs and wild boars) and gives further evidence for host selection pressures on CSFV to shaping the similarity of some synonymous codon usage with its host to some degree. Moreover, the genetic characteristic of the BVDV strain ZM-95 is distinctive with that of the rest strains of BVDV isolated from cattle suggesting that the overall codon usage pattern of the same virus can be affected within the environments of different susceptible animals.

From these results, the range of natural susceptible animals might be part of the selection pressure changing the codon usage of pestiviruses.

However, the codon distribution of the three pestiviruses is generally consistent with the overall codon usage pattern of the three livestock should not be ignored since the genetic characteristic indicates that the three viruses can replicate themselves in the hosts. Like BVDV and BDV, CSFVs can replicate in experimentally inoculated cattle (Loan and Storm, 1968) and caused reproductive disease in experimentally infected goats (Shimizu and Kumagai, 1989).

Moreover, comparing with a variety of cattle syndromes caused by BVDV, natural infection syndromes of domestic pigs has been subclinical infection and usually associated with a disease similar to mild CSF (Liess and Moennig, 1990; Terpstra and Wensvoort, 1998). These results indicate that the relationship of the overall codon usage pattern between the heterologous genes and the target host cells should be analyzed to assess the translation rates of the heterologous genes in the target cells.

## CONCLUSION

Generally, the genetic diversity of CSFV is different with BVDV and BDV in the overall codon usage pattern, implying the existence of difference in their evolutionary and epidemiological dynamics. Since, the natural susceptible animals to which CSFV can only infect is domestic pigs and wild boars, the genetic diversity of CSFV might be different from BVDV and BDV which have a large range of susceptible animals. Additionally, non-convergent trend of the BVDV strain ZM-95 in the overall codon usage pattern away from the rest of BVDV strains isolated from cattle indicates that the selection from distinctive susceptible animals is influencing on the overall codon usage of BVDV. Hence, for the nucleotide composition of pestiviruses, the overall codon usage pattern and codon distribution reflected by the translation selection from host are at least explained by the equilibrium between mutation pressure and translation selection.

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## REFERENCES

- Aragones, L., A. Bosch and R.M. Pinto, 2008. Hepatitis A virus mutant spectra under the selective pressure of monoclonal antibodies: Codon usage constraints limit capsid variability. *J. Virol.*, 82: 1688-1700.
- Aragones, L., S. Guix, E. Ribes, A. Bosch and R.M. Pinto, 2010. Fine-tuning translation kinetics selection as the driving force of codon usage bias in the hepatitis A virus capsid. *PLoS Pathogens*, Vol. 6, No. 3. 10.1371/journal.ppat.1000797.
- Bahir, I., M. Fromer, Y. Prat and M. Linial, 2009. Viral adaptation to host: A proteome-based analysis of codon usage and amino acid preferences. *Mol. Syst. Biol.*, Vol. 5. 10.1038/msb.2009.71.
- Becher, P., M. Orlich, A.D. Shannon, G. Homer, M. Konig and H.J. Thiel, 1997. Phylogenetic analysis of pestiviruses from domestic and wild ruminants. *J. Gen. Virol.*, 78: 1357-1366.
- Carbrey, E.A., W.C. Stewart, J.I. Kresse and M.L. Snyder, 1976. Natural infection of pigs with bovine viral diarrhea virus and its differential diagnosis from hog cholera. *J. Am. Vet. Med. Assoc.*, 169: 1217-1219.
- Coleman, J.R., D. Papamichail, S. Skiena, B. Futcher, E. Wimmer and S. Mueller, 2008. Virus attenuation by genome-scale changes in codon pair bias. *Science*, 320: 1784-1787.
- Cutter, A.D., J.D. Wasmuth and M.L. Blaxter, 2006. The evolution of biased codon and amino acid usage in nematode genomes. *Mol. Biol. Evol.*, 23: 2303-2315.
- Drake, J.W., 1993. Rates of spontaneous mutation among RNA viruses. *Proc. Natl. Acad. Sci. USA.*, 90: 4171-4175.
- Duret, L. and L.D. Hurst, 2001. The elevated GC content at exonic third sites is not evidence against neutralist models of isochore evolution. *Mol. Biol. Evol.*, 18: 757-762.
- Gustafsson, C., S. Govindarajan and J. Minshull, 2004. Codon bias and heterologous protein expression. *Trends Biotechnol.*, 22: 346-353.
- Karlin, S., B.E. Blaisdell and G.A. Schachtel, 1990. Contrasts in codon usage of latent versus productive genes of Epstein-Barr virus: Data and hypotheses. *J. Virol.*, 64: 4264-4273.
- Karlin, S., W. Doerfler and L.R. Cardon, 1994. Why is CpG suppressed in the genomes of virtually all small eukaryotic viruses but not in those of large eukaryotic viruses? *J. Virol.*, 68: 2889-2897.
- Kawashima, Y., K. Pfafferoth, J. Frater, P. Matthews, R. Payne and M. Addo *et al.*, 2009. Adaptation of HIV-1 to human leukocyte antigen class I. *Nature*, 458: 641-645.
- Knight, R.D., S.J. Freeland and L.F. Landweber, 2001. Rewiring the keyboard: Evolvability of the genetic code. *Nat. Rev. Genet.*, 2: 49-58.
- Laddomada, A., 2000. Incidence and control of CSF in wild boar in Europe. *Vet. Microbiol.*, 73: 121-130.
- Liess, B. and V. Moennig, 1990. Ruminant pestivirus infection in pigs. *Rev. Sci. Tech.*, 9: 151-161.
- Liu, Y.S., J.H. Zhou, H.T. Chen, L.N. Ma, Y.Z. Ding, M. Wang and J. Zhang, 2010. Analysis of synonymous codon usage in porcine reproductive and respiratory syndrome virus. *Infect. Genet. Evol.*, 10: 797-803.
- Liu, Y.S., J.H. Zhou, H.T. Chen, L.N. Ma, Z. Pejsak, Y.Z. Ding and J. Zhang, 2011. The characteristics of the synonymous codon usage in enterovirus 71 virus and the effects of host on the virus in codon usage pattern. *Infect. Genet. Evol.*, 11: 1168-1173.
- Loan, R.W. and M.M. Storm, 1968. Propagation and transmission of hog cholera virus in nonporcine hosts. *Am. J. Vet. Res.*, 29: 807-811.
- Nakamura, Y., T. Gojobori and T. Ikemura, 2000. Codon usage tabulated from international DNA sequence databases: Status for the year 2000. *Nucleic Acids Res.*, 28: 292-292.
- Nettleton, P.F., J.A. Gilray, P. Russo and E. Dliissi, 1998. Border disease of sheep and goats. *Vet. Res.*, 29: 327-340.

- Plotkin, J.B., H. Robins and A.J. Levine, 2004. Tissue-specific codon usage and the expression of human genes. *Proc. Natl. Acad. Sci. USA.*, 101: 12588-12591.
- Roehe, P.M., M.J. Woodward and S. Edwards, 1992. Characterisation of p20 gene sequences from a border disease-like pestivirus isolated from pigs. *Vet. Microbiol.*, 33: 231-238.
- Sanchez, G., A. Bosch and R.M. Pinto, 2003. Genome variability and capsid structural constraints of hepatitis A virus. *J. Virol.*, 77: 452-459.
- Santos, M.A., G. Moura, S.E. Massey and M.F. Tuite, 2004. Driving change: The evolution of alternative genetic codes. *Trends Genet.*, 20: 95-102.
- Sharp, P.M., T.M.F. Tuohy and K.R. Mosurski, 1986. Codon usage in yeast cluster-analysis clearly differentiates highly and lowly expressed genes. *Nucleic Acids Res.*, 14: 5125-5143.
- Shimizu, M. and T. Kumagai, 1989. Experimental infection of pregnant goats with swine fever virus. *Vet. Microbiol.*, 20: 207-214.
- Sugiyama, T., M. Gursel, F. Takeshita, C. Coban and J. Conover *et al.*, 2005. CpG RNA: Identification of novel single-stranded RNA that stimulates human CD14<sup>+</sup>CD11c<sup>+</sup> monocytes. *J. Immunol.*, 174: 2273-2279.
- Terpstra, C. and G. Wensvoort, 1998. Natural infections of pigs with bovine viral diarrhoea virus associated with signs resembling swine fever. *Res. Vet. Sci.*, 45: 137-142.
- Vilcek, S. and P.F. Nettleton, 2006. Pestiviruses in wild animals. *Vet. Microbiol.*, 116: 1-12.
- Wong, E.H., D.K. Smith, R. Rabadan, M. Peiris and L.L. Poon, 2010. Codon usage bias and the evolution of influenza A viruses. Codon usage biases of influenza virus. *BMC Evol. Biol.*, Vol: 10, 10.1186/1471-2148-10-253.
- Zhi, N., Z. Wan, X. Liu, S. Wong, D.J. Kim, N.S. Young and S. Kajigaya, 2010. Codon optimization of human parvovirus B19 capsid genes greatly increases their expression in nonpermissive cells. *J. Virol.*, 84: 13059-13062.
- Zhou, J., W.J. Liu, S.W. Peng, X.Y. Sun and I. Frazer, 1999. Papillomavirus capsid protein expression level depends on the match between codon usage and tRNA availability. *J. Virol.*, 73: 4972-4982.
- Zhou, J.H., J. Zhang, H.T. Chen, L.N. Ma and Y.S. Liu, 2010. Analysis of synonymous codon usage in foot-and-mouth disease virus. *Vet. Res. Commun.*, 34: 393-404.
- Zhou, J.H., Z.L. Gao, J. Zhang, H.T. Chen and Z. Pejsak *et al.*, 2012. Comparative the codon usage between the three main viruses in pestivirus genus and their natural susceptible livestock. *Virus Genes*, 44: 475-481.