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Comparative genomic variation in porcine species could lead to species-specific responses to ASF

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Target Audience: Researchers, Animal health workers

Abstract

African Swine Fever (ASF) is a highly contagious viral disease of pigs and is of utmost concern due to its presence in list A diseases by the United Nations Office International des Epizooties (OIE). It causes significant economic losses in affected countries due to resulting high mortality rates associated. In contrast, the porcine species endogenous to Africa tolerate infection. The ability of the virus to persist in one host, while killing another genetically related host implies that the disease may influence by the hosts genetic variation. This study identify genetic polymorphism among three suidae family species in susceptibility to ASFV based on five genes such as RELA, NFATCI, PPIA, PPP3CB, NKBIA. It also predicts the protein structure of each gene and draw phylogenetic trees of each gene using human as an out-group. The nucleotide and amino acid sequence (AAS) of the 5 genes of pig, warthog and Babyrus were downloaded from the National Center for Biotechnology information (NCBI) data base, United State of America and Universal protein resources (Uniprot) database, United Kingdom. Data and phylogenetic trees were constructed using MEGA7. The variation observed at position 531 in RELA gene between warthog and domestic pig may be a significant factor behind the susceptibility or tolerance to ASFV. This study could give genomic bases to the discovery of a potent vaccine for prevention, control and prophylaxis.

Key words: African Swine Fever, Pigs, Warthogs, Babirusa

Description of Problem

The African swine fever (ASF) is one of the most deadly viral disease affecting swine production, and has no easy cure and vaccine for prevention and control. It causes a devastating effect of about 100 % mortality in pigs (1). This plague is caused by the African swine fever (ASF) virus, which is a large deoxyribonucleic virus belonging to Asfarviridae family (2). It is noted that previously the ASFV was classified as an indovirus based on visual morphological classification. Increasing molecular biological evidences had led to its reclassification as the sole member of a new DNA virus family, Asfarviridae (Asfar-African swine fever and related viruses). African swine fever virus (ASFV) replicates in the cytoplasm and has variable virulence in domestic pigs, with infections ranging from highly lethal to subclinical. ASFV genes encoding proteins which modulate host immune response, viral virulence to domestic swine, and the ability of ASFV to replicate and spread in its tick vector. ASFV is the only known DNA-arbovirus. It is endemic across much of Africa, the disease poses a wider threat to global food security. No vaccine and the current control methods are by

diagnosis and slaughter (3).

ASFV persistently infects ticks of the genus ornithodoros from which ASFV can be isolated 5-10 years post infection (4). ASFV infection of domestic swine results in several disease forms, ranging from highly lethal to subclinical depending on contributing viral and host factors.

There are three A238L target proteins namely, RELA (v-rel avian reticuloendotheliosis viral oncogene homolog A), PPP3CB (*Protein* Phosphatase 3 Catalytic Subunit Beta), and PPIA (Peptidylprolyl isomerase A), and there are two proteins it mimics, NFKBIA (Nuclear Factor of Kappa Light Chain Gene Enhancer in B Cells Inhibitor, Alpha) and NFATC1 (Nuclear factor of activated T-cells, cytoplasmic 1), as candidates for the genetic variation between pig species that lead to species-specific responses to ASFV infection.

This study aim to identify genetic polymorphism among the different pig species in susceptible to ASFV and would aid in deduce the protein structure of each gene and to draw phylogenetic tree of each gene using human as an out-group.

Materials and Methods

Retrieval of amino acids sequences

The nucleotide and amino acid sequences (AAS) of five genes of 3 species of Suidae family namely- domestic pig, Warthog and Babirusa were free accessed from the National Center for Biotechnology information (NCBI) data base (http:// www. ncbi. nlm. nih. gov/protein/), United State of America and Universal protein resources (Uniprot) database (http:// www. uniprot .org/protein/), United Kingdom.

Determination of Percentage Identity and Similarity

The Identity and Similarity percentage of AAS of these 5 genes of these 3 species of Suidae family were identified by conducting pairwise comparison of their AAS using two or more sequences of basic local alignment search tool (BLAST).

Phylogenetic analysis

The phylogenetic tree was drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary history was inferred by using the Maximum Likelihood method based on the JTT matrix-based model (5). The tree with the highest log likelihood (-1182.1917) was shown. The percentage of trees in which the associated taxa clustered together is shown next to the branches. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using a JTT model, and then selecting the topology with superior log likelihood value. The tree was shown as being drawn to scale, with branch lengths measured in the number of substitutions per site. The analysis involved 25 amino acid sequences. All positions containing gaps and missing data were eliminated. There were a total of 171 positions in the final dataset. Evolutionary analyses were conducted in MEGA7 (6).

Prediction of Protein Structure

The AAS of these 5 proteins were submitted into phyre2 online (http:// www. sbg.bio.ic.ac.uk/phyre2/html/page.cgi) for protein structure prediction and analysis. The protein parameters (physical and chemical properties) of the 5 proteins were obtained from Expasy Bioinformatics resource portals where Protfam tool was used to obtain the protein parameters.

Results

Polymorphic differences among the three species

In RELA, the polymorphic variations

were observed at two sites at sequence 448 and 485 in domestic pig (DP) where there was insertion of Threonine (T) instead of Alanine (A) in other species and insertion of Serine (S) instead of Proline (P) in other species. The polymorphic variation was observed in Warthog at only one site at sequence 531 where there was insertion of P instead of S. In PPIA, among the species only babyrusa was different at sequence 107 and 141 where there was an insertion of A instead of T and insertion of S instead of A. In PPP3CB there was polymorphic variants observed between the domestic and the Warthog at 396 AA sequence position where there was insertion of V instead of E in pig. Deletion from 395-405 of amino acid (AA) sequence of RLIYGNKKV were considered where they were absent or deleted in both Warthog and Babirusa, therefore it was only pig and human that has those AA sequences. In NFKBIA, the Babyrusa variation was observed at sequence 170 where there was an insertion of E instead of Q. In NFATC1, there was polymorphism at AA sequence position 278 where there was an insertion of Phenylalanine (F) instead of S in domestic pig. Also at AA sequence 297 in Babirusa, where there was an insertion of Isoleucine (I) instead of V.

Phylogenetic tree of each gene in the suidae family

The organisms with their accession numbers, amino acid sequence (AAS) length of each gene that was retrieved from Uniprot were shown in Table 1a-1e. There was high percentage identity (100-97.6%) and similarity (100-97.6%) between the pig and other two suidae family. The NKBIA gene, had the highest percentage identity and similarity (100%), followed by PPIA, RELA, NFATC1 and PP3CB gene. The variations of AASL were observed among the 3 suidae family for each gene as shown in Table 1a-1e. The 3 species had the same AAS length only in PPIA and NFKBIA genes while in other genes there were a lot of variations in AASL among the 3 species. The e-values were highly significant in all the five genes for all the 3 species ranging from (5e-178 to 0.00) as shown in Table 1a-1e.

Table 1a: Alignment results, physical and chemical properties of RELA gene/protein

Protein Parameters	Pig	Warthog	Babirusa
Accession no	F2QA75	F2QA76	F2QA77
Identity %	100	99.5	98.2
Similarity %	100	99.5	98.9
e-value	0.00	0.00	0.00
AASL	551	551	549
Carbon	2654	2657	2641
Hydrogen	4175	4177	4157
Nitrogen	745	745	741
Oxygen	813	810	812
Sulphur	19	19	19
Total atom	8406	1538	8370
Instabilit Index	50.21	50.29	51.84
Stability	Unstable	Unstable	Unstable
Aliphatic index	72.99	73.18	73.61
GRAVY	-0.451	-0.450	-0.447
N-terminal Hydropathicity	Asparagine	Asparagine	Asparagine

Table 1b: Alignment results, physical and chemical properties of PP3CB gene/protein

Protein Parameters	Pig	Warthog	Babirus
Accession no	F2Q9B8	F2Q9B9	F2Q9C0
Identity %	100	97.6	99.8
Similarity%	100	97.6	99.8
E-value	0.00	0.00	0.00
AASL	481	492	481
Carbon	2431	2487	2437
Hydrogen	3762	3850	3766
Nitrogen	666	678	666
Oxygen	717	736	718
Sulphur	26	25	25
Total atom	7602	7776	7612
Instability Index	44.42	44.37	44.42
Stability	Unstable	Unstable	Unstable
Aliphatic index	82.10	82.85	82.10
GRAVY	-0.289	-0.284	-0.297
N-terminal	Proline	Proline	Proline

Table 1c: Alignment results, physical and chemical properties of PPIA gene/protein

Protein Parameters	Pig	Warthog	Babirusa
Accession no	F2Q9A3	F2Q9A4	F2Q9A5
Identity %	100	100	98.8
Similarity %	100	100	99.4
E-value	2e-118	2e-118	3.3e-117
AASL	164	164	164
Carbon	769	796	795
Hydrogen	1227	1227	1225
Nitrogen	217	217	217
Oxygen	234	234	234
Sulphur	9	9	9
Total atom	2483	2483	2480
Instability Index	10.23	10.23	10.23
Stability	Stable	Stable	stable
Aliphatic index	61.83	6.183	61.83
GRAVY	-0.293	-0.450	-2.93
N-terminal	Methionine	Asparagine	Methionine

Table 1d: Alignment results, physical and chemical properties of NFKBIA gene/protein

Protein Parameters	Pig	Warthog	Babirusa
Accession no	F2Q9A6	F2Q9A7	F2Q9A8
Identity %	100	100	100
Similarity %	100	100	100
E-value	2.4e-30	2.4e-30	2.4e-30
AASL	40	40	40
Carbon	2654	2657	5322
Hydrogen	4175	4177	8376
Nitrogen	745	745	1498
Oxygen	813	810	1637
Sulphur	19	19	38
Total atom	8406	1538	16871
Instability Index	50.21	50.29	43.62
Stability	Unstable	Unstable	Unstable
Aliphatic index	72.99	73.18	72.61
GRAVY	-0.451	-0.450	-0485
N-terminal	Asparagine	Asparagine	Methionine

Table 1e: Alignment results, physical and chemical properties of NFATC1 gene/protein

Protein Parameters	Pig	Warthog	Babirusa
Accession no	F2Q9B	F2Q9B4	F2Q9B6
Identity %	100	98.8	99.6
Similarity %	100	99.2	100
e-value	0,0	5.5e-178	2.8e-179
AASL	246	250	245
Carbon	1112	1128	1109
Hydrogen	1715	1742	1711
Nitrogen	323	328	323
Oxygen	370	379	367
Sulphur	6	7	6
Total atom	3526	1538	3516
Instability Index	78.13	78.53	78.75
Stability	Unstable	Unstable	Unstable
Aliphatic index	57.56	57.04	58.20
GRAVY	-0.520	-0.534	-0.518
N-terminal	Isoleucine	Isoleucine	Isoleucine

The phylogenetic trees of each five genes showing the relationship between the 3 species of Suidae family were shown in Figure 1a-1e. The phylogenetic relationships between these 3 species in each of the 5 genes were shown an optimal tree with the sum of branch length of 0.1886. The percentage of replicate trees in which the associated taxa clustered together in

the bootstrap tests (1000 replicates) were shown next to the branches. The trees showed that the 5 genes have two clades where the first clade contained the suidae family while the second clade contained the non-suidae family (Human) which was an out group.

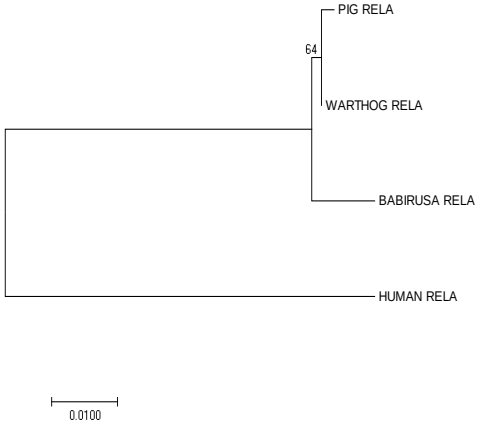


Figure 1a: Phylogenetic tree of RELA gene

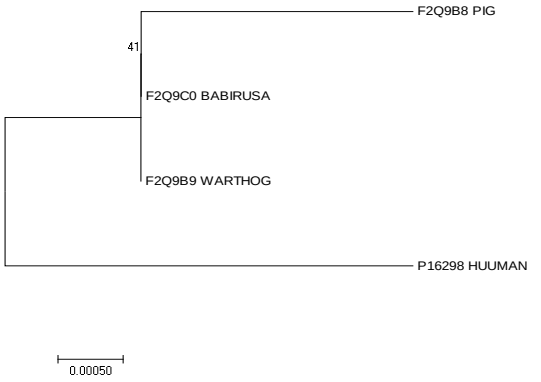
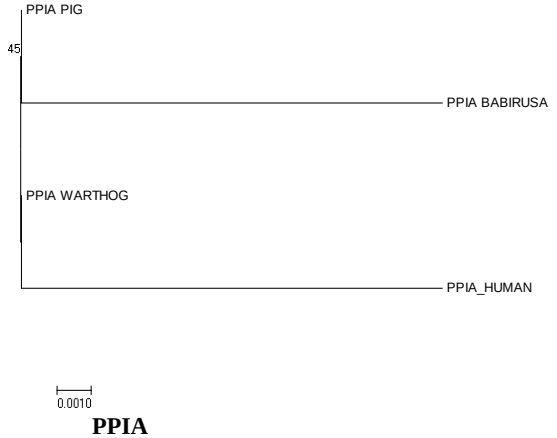
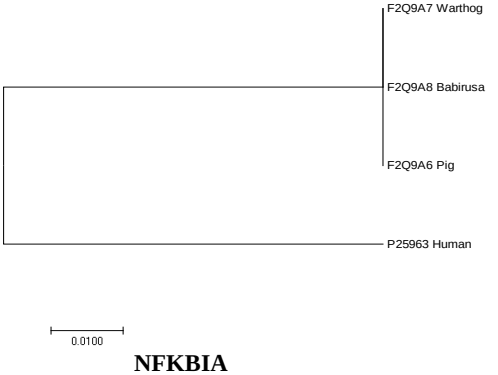


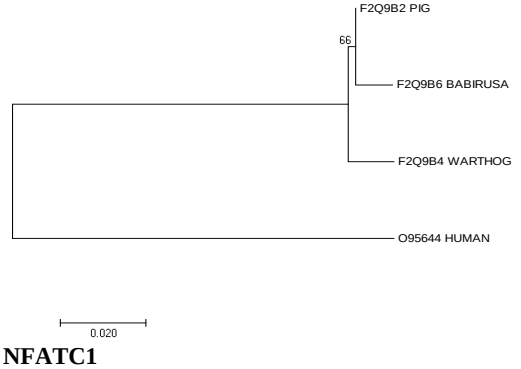
Figure 1b: Phylogenetic tree of PP3CB gene



PPIA



NFKBIA



NFATC1

Physical properties of the 5 proteins

The variation was observed in all the protein parameters for all the proteins among all the species. The N–terminal of RELA protein was Asparagine in all the species, they were unstable and GRAVY negative as shown in Table 1a above. From the 61% residues modelled at more than 90% of RELA pig predicted (Figure 1a), only 43% can be meaningfully predicted (4). Therefore the overall confidence was considered to be low (<70%) and no binding site can be predicted. The result as shown below (Figure 2a) can be viewed at http:// www. sbg.bio. ic.ac. uk/phyre 2/phyre2_output/.

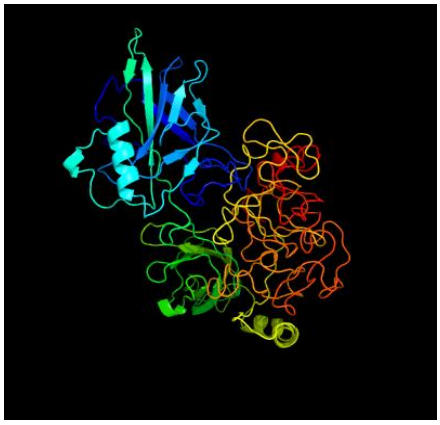


Figure 2a: RELA Protein structure

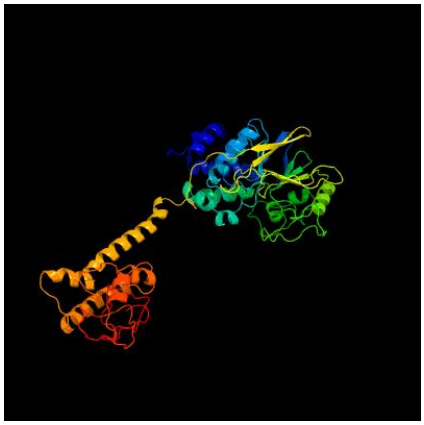


Figure2b: PP3CB protein structure of pig.

N-terminal of PP3CB protein for all the species was Proline, they were unstable and GRAVY –negative as shown in Table 1b. The final model of PP3CB protein structure (Figure 2b) was 80% modelled at >90% confidence and submitted on line (3DLigandsite) to predict potential binding sites of the protein where the binding sites contacts were Asparagine, Aspartic acid and Histidine.

N-terminal of PPIA protein of all the species were methionine with the exception of warthog that was Asparagine, they were all stable and GRAVY-negative as shown in Table 1c. The overall confidence in the final model of protein was 0% modelled at >90% was considered too low (<70%) for ligand site prediction. The N-terminal of NFKBIA protein was Asparagine for pig and Warthog while it was Methionine for Babirusa as shown in Table 1d above. They were all unstable and GRAVY –negative. The overall confidence in the final model of protein was 0% modelled at >90% confidence) was considered too low (<70%) for ligand site prediction.

N-terminal of NFATC1 protein was Isoleucine for all the species, they were all unstable and GRAVY –negative as shown in Table 1e. The overall confidence in the final model of protein was 0% modelled at >90%) was considered too low (<70%) for ligand site prediction.

Discussion

In the domestic pig the variation that occurred from the results really showed differences from the reservoir host, (Warthog). The polymorphic variation that was observed in warthog at only sequence 531, where there was insertion of P instead of S, correlates with the phosphorylation site in transactivation domain 1 (Position 531) as reported by (7). The function of approximately one-third of all

eukaryote proteins that is controlled by a process called phosphorylation. The detected P (proline) sequence at position 531 instead of S (serine), represent an intriguing candidate regulator for the reduced pathogenicity observed in domestic pigs infected with ASFV as reported by 8; 7.

The deletion of nine (9) amino acids RLIYGNKKV at position 395-405, in Babyrusa and warthog, does not correlate with the findings of (8) where it was only six AA deletion in Babyrusa and Warthog.

There were high conserved regions observed in all the three species (domestic pig and warthog) in all the three genes (PPIA, NFATC1, PPP3CB, NFKBIA and RELA), that shows complete homology at the translated protein level among the species. This observation was in agreement with (9).

The variations observed between the PPP3CB warthog and Babyrusa, where the warthog contained one insertion of valine (V) instead of glutamic acid (E) reflected the long evolutionary distance that existed between these species as reported by (10).

Conclusion and Applications

1. There were many polymorphic variations observed among these 3 suidae families in all the 5 genes but the most significant difference was observed in Pig and warthog RELA gene at the 531 position which was phosphorylation site. This may be the reason for susceptibility of domestic pigs to African swine fever and resistance of warthog to it.
2. Further studies are still going on but this result shows that these variations between the pig and warthog can be the factor why pig are susceptible to ASFV and warthog resistant to ASFV.
3. This study could give genomics bases to the discovery of a potent vaccine for prevention control and prophylaxis.

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