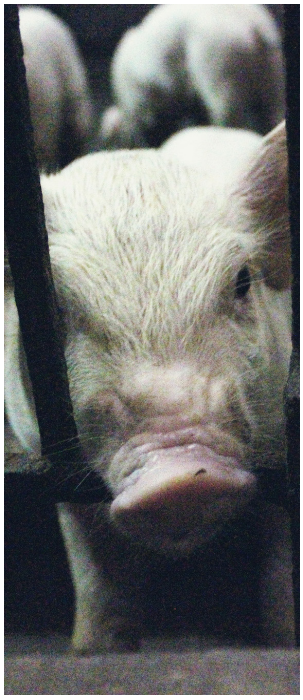

ILRI RESEARCH BRIEF 86
April 2018

Sequencing swine leucocyte alleles for vaccine development

Lucilla Steinaa, Sonal Henson, Soren Buus, Annette Stryhn, Morten Nielsen, Nicholas Svitek, Cynthia Onzere, Edwina Bochere, Gideon Ndambuki and Richard Bishop

Why is this work important?

Swine leucocyte antigen (SLA) genes are among the most important determinants of swine immune responses to disease and vaccines. Accurate and comprehensive SLA genotyping methods are required to understand how SLA gene polymorphisms affect immunity, especially in pigs with diverse genetic backgrounds. The work outlined in this brief contributes to advances in knowledge required for generation of efficient vaccines against swine pathogens and diseases, such as African swine fever.

African swine fever (ASF) is a haemorrhagic fever affecting pigs. If introduced into a farm, it can kill all the animals within a few days. The disease is caused by a large DNA virus and the only member of Asfaviridae family, but has similarities with the pox viruses, such as chicken pox and goat pox, among others. The virus is initially found in cells of the macrophage type; but in the later phases of the disease, it also infects other cell types such as epithelial cells. Distinct clinical symptoms are high fever, redness (hemorrhage) of the ears and on other patches of the skin, and death. Unfortunately, there are no available commercial vaccines or treatment to control or prevent ASF virus infection. The only method of preventing ASF is by using strict biosecurity methods, e.g. fencing of animals, avoiding contact with wild pigs, washing of boots before entering pig enclosures, use of quarantine pens for new animals, etc. Compartmentalization and zoning of pig herds can be used to keep areas ASF-free; however, this approach can be difficult to introduce and maintain on a large-scale in Africa as pigs often roam freely in rural and peri-urban systems. Moreover, poor African pig producers are less likely to implement control strategies or report disease outbreaks because of a lack of knowledge and incentives to do so. Therefore, this disease poses serious socio-economic consequences in affected and nearby countries and highlights the urgency of developing efficient countermeasures against ASF.

With an estimated 34 million pigs in sub-Saharan Africa, an ASF vaccine could benefit from 6–17 million smallholder farmers, providing protection in cases of generalized outbreaks and preventing outbreaks in nearby areas. As the pig population in Africa rises, disease spread could be increasingly problematic (Steinaa et al. 2016).

In the 1960s, ASF became endemic in Spain and Portugal and complete eradication took more than 30 years. In 2007, Africa swine fever was introduced into the Caucasus region of Eurasia, where it has spread widely among wild boar and domesticated pigs. This virus has caused outbreaks in pigs as far west as the easternmost countries of the European Union, and it has also been detected in wild boar in Iran. The disease now threatens western Europe, China and the Far East (CFSPH et al. 2015).

What are Swine leukocyte antigens?

SLAs are tissue type antigens in pigs, also more commonly known known as major histocompatibility complex molecules (MHC). These antigens are very diverse and vary among individual pigs. The diversity among the different molecules are clustered in a certain region of the molecules. This region is responsible for binding of short peptide fragments from pathogens, which they present to cells from the immune system. This can then trigger the immune system to react to the pathogen to clear it from the body.

SLA class I molecules are encoded by three regions in the pig genome, SLA-1, SLA-2 and SLA-3 (Lunney et al. 2009; Renard et al. 2006). SLAs from European pigs are characterized far better than their African counterparts. Currently, 85 SLA-1, 85 SLA-2 and 36 SLA-3 gene sequences, coding for MHC class I molecules, have been published in the Immuno Polymorphism Database , but very few, if any, originate from African pigs. The origin of African pigs is not completely clear, and there is phylogenetic divergence between pigs from West and East Africa. Pigs from East Africa have high frequencies of genes from pigs in the Far East, consistent with data found from African chickens, which confirms that livestock were transported from Far East over the Indian Ocean thousands of years ago. Later, with successive European colonizations, other pig breeds were introduced and were mixed with the original pigs, further adding to the gene pool (Amills et al. 2013).

Why are SLA sequences interesting?

Each SLA type binds pathogen peptides with a particular peptide motif, e.g. the amino acid arginine at position 2 and lysine at position 9. Different SLA types exhibit a preference for different peptides. Hence, the SLA molecules 'fit' with certain peptides in the pathogen, also called epitopes. This is important knowledge for the development of subunit vaccines. In the process of selecting antigens/epitopes, for them to be included in a subunit vaccine, consideration must be given to the SLAs in the target pig population. Knowledge of peptide motifs can also be used for prediction of epitopes using neural network algorithms such as NetMHCpan (Hoof et al. 2009), developed by Danish Technical University (Stryhn et al. 1996).

ILRI sequencing study–findings and next steps

ILRI has sequenced the expressed swine leucocyte antigens from 34 Kenyan pigs. Messenger RNA, which represents expressed genes, was copied to DNA (cDNA) and parts of the SLA genes were amplified and sequenced using Illumina MiSeq. Many novel sequences were identified.

Three African SLAs have been expressed as protein and positional scanning combinatorial peptide library analyses have been performed by our Danish collaborators, Soren Buus and Anette Stryhn (University of Copenhagen). This has generated peptide binding motifs for these three SLA molecules (Morten Nielsen, Danish Technical University).

The expressed SLAs can now be used to make reagents to study the specificity of cellular immune responses to ASF, thereby accelerating vaccine development research.

References

Amills, A., Ramirez, O., Galman-Omitogun, O. and Clop, A. 2013. Domestic Pigs in Africa. *African Archaeological Review* 30(1):73–82. doi:10.1007/s10437-012-9111-2

Centre for Food Security and Public Health (CFSPH) and Institute for International Cooperation in Animal Biologics (IICAB). 2015. *African Swine fever factsheet*. Accessed at http://www.cfsph.iastate.edu/Factsheets/pdfs/african_swine_fever.pdf on 26 March 2018.

Hoof, I., Peters, B., Sidney, J., Pedersen, L.E., Sette, A., Lund, O., Buus, S. and Nielsen, M. 2009. NetMHCpan, a method for MHC class I binding prediction beyond humans. *Immunogenetics* 61(1):1-13. doi: 10.1007/s00251-008-0341-z.

Immuno Polymorphism Database https://www.ebi.ac.uk/ipd.

Lunney, J.K., Ho, C.S., Wysocki, M. and Smith, D.M. 2009. Molecular genetics of the swine major. histocompatibility complex, the SLA complex. *Dev Comp Immunol* 33:362–74. doi: 10.1016/j.dci.2008.07.002.

Renard, C., Hart, E., Sehra, H., Beasley, H., Coggill, P., Howe, K. et al. 2006. The genomic sequence and analysis of the swine major histocompatibility complex. *Genomics* 88:96–110. doi:10.1016/j.ygeno.2006.01.004.

Steinaa, L., Bishop, R., Okoth, E., Svitek, N. and Riitho, V. 2016. *Pig vaccines and diagnostics for African swine fever in Uganda*. Livestock and Fish Brief 26. Nairobi: ILRI.

Stryhn, A., Pedersen, L.O., Romme, T., Holm, C.B., Holm, A. and Buus, S. 1996. Peptide binding specificity of major histocompatibility complex class I resolved into an array of apparently independent subspecificities: quantitation by peptide libraries and improved prediction of binding. *Eur J Immunol*. 26(8):1911-8.

Support and collaborators

This work was undertaken as part of the research project Towards a vaccine against African swine fever, which was funded by the German BMZ with co-funding from the CGIAR Research program on Livestock and the CGIAR fund donors. This project was undertaken in collaboration with Friedrich Loeffler Institute in Riems, the University of Copenhagen and the Danish Technical University.

Photo credits:
Page 1: (left)ILRI/Hanh L (right) ILRI/Mann
Page 2: (top) ILRI/Charlie Pye-Smith
(bottom) ILRI/Jules Mate
Page 3: ILRI/Stevie Mann

Lucilla Steinaa, Sonal Henson, Nicholas Svitek, Edwina Bochere and Gideon Ndambuki work for the International Livestock Research Institute, Soren Buus and Annette Stryhn at the University of Copenhagen, Morten Nielsen at the Danish Technical University, and Richard Bishop and Cynthia Onzere for Washington State University.

Contact

Lucilla Steinaa
ILRI, Kenya
l.steinaa@cgiar.org
ilri.org

Federal Ministry for Economic Cooperation and Development
ILRI INTERNATIONAL LIVESTOCK RESEARCH INSTITUTE
CGIAR RESEARCH PROGRAM ON Livestock

ILRI thanks all donors that globally support its work through their contributions to the CGIAR system

Patron: Professor Peter C Doherty AC, FAA, FRS
Animal scientist, Nobel Prize Laureate for Physiology or Medicine–1996

Box 30709, Nairobi 00100 Kenya
Phone +254 20 422 3000
Fax +254 20 422 3001
Email ilri-kenya@cgiar.org

ilri.org
better lives through livestock

ILRI is a CGIAR research centre

Box 5689, Addis Ababa, Ethiopia
Phone +251 11 617 2000
Fax +251 11 667 6923
Email ilri-ethiopia@cgiar.org

ILRI has offices in East Africa • South Asia • Southeast and East Asia • Southern Africa • West Africa

 April 2018