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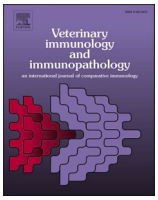

Multiepitope array as the key for African Swine Fever diagnosis

Bruno Tilocca [a], Viviana Greco [b,c], Alessio Soggiu [d], Andrea Urbani [b,c], Domenico Britti [a,e], Luigi Bonizzi [d], Canio Buonavoglia [f], Paola Roncada [a,*]

[a] Department of Health Science, University "Magna Graecia" of Catanzaro, Viale Europa, 88100, Catanzaro, Italy
[b] Department of Basic Biotechnological Sciences, Intensivological and Perioperative Clinics, Università Cattolica del Sacro Cuore, Largo F. Vito 1, 00168 Rome, Italy
[c] Fondazione Policlinico Universitario Agostino Gemelli, Largo A. Gemelli, 8-00168 Rome, Italy
[d] Department of Biomedical, Surgical and Dental Sciences, University of Milano, Via Celoria n.10, 20133 Milano, Italy
[e] Interdepartmental Center of Veterinary Services (CIS), University "Magna Graecia" of Catanzaro, Viale Europa, 88100, Catanzaro, Italy
[f] Department of Veterinary Medicine, University of Bari, SP per Casamassima Km 3, 70010, Valenzano, Italy

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ABSTRACT

African Swine Fever (ASF) is an acute hemorrhagic fever affecting suids with high mortality and morbidity rate. The causal agent of ASF, the African Swine Fever Virus (ASFV), is an icosahedral virus of 200 nm diameter, composed of an outer envelope layer of host derivation and a linear 170–190 kb long dsDNA molecule. As of today, no efficient therapeutic intervention nor prophylactic measures exist to fight ASFV diffusion, underlining the importance of the early diagnosis and the need for efficient in-field screening of ASF. Recommended guidelines for the diagnosis of ASF are unpracticable in the desirable context of the rapid in-farm screening. In this view, the design of innovative diagnostics based on a panel of multiple ASFV epitopes would amend versatility and the analytical performances of the deliverable, ensuring high quality and accuracy standards worth of implementation in rapid in-field monitoring programs. Pursuing this view, we performed epitope prediction from the major AFSV structural proteins holding the potential to be targeted in innovative rapid diagnostic tests. Selected ASFV structural protein sequences were retrieved from data repositories and their tridimensional structure was computed. Linear and 3D protein structures were subjected to the prediction of the epitope sequences, that are likely to elicit antibody production, by independent bioinformatic tools, providing a list of candidate biomarkers whose batch employment held the potential suitability for the unbiased rapid in-field diagnosis and, in turn, might be implemented in screening programs, crowing the current monitoring and control campaigns that are currently running worldwide.

1. Introduction

African Swine Fever (ASF) is an acute hemorragic fever affecting wild suids and domestic pigs with dramatically high mortality and morbility rate (Dixon et al., 2019; Trotta et al., 2022). The causal agent of ASF, the African Swine Fever Virus (ASFV), is an icosahedral virus of 200 nm diameter, composed of an outer envelope layer of host derivation. This surrounds the viral capsid, the inner envelope and the core-shell. The viral genome is a linear 170–190 kb long dsDNA molecule encoding 150–200 viral proteins; of these, 68 proteins have structural functions whilst more than 100 proteins have been identified with replicative/virulence functions (Alejo et al., 2018; Alonso et al., 2018; Arias et al., 2002) (Fig. 1). Nowadays, 24 different genotypes are described on the basis of the structural protein sequence (Sánchez-Vizcaíno et al., 2012). Two major genotypes are predominantly detected on a global scale: genotype I, *i.e.* the ancestral genotype, now endemic in Africa and Sardinia and the genotype II which is typically observed in the Asian and Western Europe countries (Cwynar et al., 2019; Dixon et al., 2019). ASFV persists for a very long time in an array of biological sample kinds such as slurry, feces, serum, tissues and blood, including the putrefied blood and animal carcasses. Moreover, the virus persists in meat and its by-products even after refrigeration/frozen, besides being highly resistant to a variety of chemicals and inactivating conditions (Mazur-Panasiuk et al., 2019; "Scientific Opinion on African swine fever | EFSA," n.d.).

Being a non-zoonotic agent, ASFV is not a direct threat to human

* Corresponding author.
E-mail addresses: tilocca@unicz.it (B. Tilocca), viviana.greco@unicatt.it (V. Greco), alessio.soggiu@unimi.it (A. Soggiu), andrea.urbani@unicatt.it (A. Urbani), britti@unicz.it (D. Britti), luigi.bonizzi@unimi.it (L. Bonizzi), canio.buonavoglia@uniba.it (C. Buonavoglia), roncada@unicz.it (P. Roncada).

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health; nevertheless, the elevated environmental resistance, persistence and diffusion make ASF one of the most economically devastating diseases in the swine producing countries, all-over the world (Dixon et al., 2019; Trotta et al., 2022). In this scenario, early diagnosis of ASF, either outbreaks or novel ASF cases in the ASFV-free regions, is of great importance for the timely acquisition of the adequate measures to contrast virus diffusion and control/eradicate the disease (Cwynar et al., 2019; Oura et al., 2013).

To date, no efficient therapeutic intervention nor prophylactic measures exist to fight ASFV diffusion, underlining the need for early in-field screening of ASF (Cwynar et al., 2019; Gallardo et al., 2015; King et al., 2011; Penrith et al., 2013). Current diagnosis recommended by the WOAH relies on a combination of methods for the direct detection of ASFV including PCR (*i.e.* the common method employed by national reference laboratories), ELISA-based detection of the viral antigens and the direct isolation of ASFV by cultivation. Indirect diagnosis can also be accomplished by either ELISA or fluorescent antibody detection, acknowledging their sensibility and specificity (Cwynar et al., 2019; King et al., 2011; Oura et al., 2013; Wang et al., 2020; Zhu et al., 2022a). Unfortunately, the above methods are rather laborious, time-consuming and expert personnel demanding, making them unpracticable in the desired context of the rapid in-farm screening. In addition, it must be beared in mind that the cases of remote and/or rural areas, that are not directly connected with the sanitary and surveillance structures, face the adjunctive issue of the further prolonged diagnosis times; resulting in a strong reduction of efficacy of the monitoring and control programs (Trotta et al., 2022). In this view, several studies are being performed to optimize the detection of ASFV exploiting cutting-edge technologies for the early diagnosis of ASF (Carlson et al., 2018; Gao et al., 2018; Sastre et al., 2016; Ye et al., 2019; Zhai et al., 2020). Nevertheless, the stability and sensitivity of these new methods still need to be further improved to reach, or overcome, the diagnostic performances of the WOAH-recommended diagnostic methods (Zhu et al., 2022a).

To the best of our knowledge, various diagnostic approaches are so far designed for the direct detection of ASFV. Each of these relies on the target of a single epitope and is subjected to specific limitations (*e.g.* ease of execution, rapidity, large scale applicability), hindering the fair implementation of these innovations in the in-field diagnosis. Moreover, among the promising alternatives, collapses of the analytical performances have been registered while facing unconventional samples and/or biological sample kinds diverse from those employed in the assessment of the diagnostic tool (Deutschmann et al., 2021). In this view, the design of innovative diagnostics based on a panel of multiple ASFV epitopes would amend versatility and the analytical performances of the deliverable, ensuring high quality and accuracy standards worth of implementation in the monitoring programs. To promote the design of innovative companion diagnostics according to this view, the present work performed epitope prediction from the major AFSV structural proteins holding the potential to be targeted by rapid diagnostic tests. These, in turn, might be implemented in in-field screening programs, crowing the current monitoring and control campaigns running in diverse countries around the globe.

2. Material and methods

2.1. Data collection and protein structure prediction

Structural proteins of the ASFV particle were selected on the basis of the likelihood of being targeted by direct diagnosis approaches through antigen-antibody specificity. Protein sequences were retrieved for each of the selected entries from UniProt KB data repositories (https://www.uniprot.org/) (Bateman et al., 2021) and, when available, the 3D structure of the molecule was obtained from the Protein Data Bank data repository (PDB, https://www.rcsb.org/) (Zardecki et al., 2022). The tridimensional structure of the proteins lacking a publicly available PDB structure has been computed *ad hoc via* SWISSMODEL (https://swissmodel.expasy.org/) (Waterhouse et al., 2018).

2.2. B-epitopes sequence prediction

Selected proteins were then subjected to *in-silico* prediction of the epitope sequences that are likely to elicit antibody production by three commonly employed tools, each of which relies on a peculiar algorithm and emphasizes specific features of the potential epitope sequences. Both linear and discontinuous epitopes were predicted for the selected proteins *via* IEDB (http://tools.iedb.org/bcell/), and DiscoTope (http://tools.iedb.org/discotope/) and the ElliPro antibody epitopes prediction tool (http://tools.iedb.org/ellipro/) (Vita et al., 2019).

2.3. Linear b-epitopes prediction

With regard to IEDB bioinformatic tool, Bepipred algorithm was chosen for the prediction of the linear protein epitopes capable of binding B-cells. This employs a combination of a hidden Markov model and a propensity scale method (Jespersen et al., 2017). Each protein residue is scored for its epitope behavior and the sole aminoacid with a score greater than or equal to 0.35 was considered as a potential epitope. Being a descriptive study, linear peptide epitopes of all lengths were considered as putative biomarkers for ASFV detection. Linear epitopes were also calculated by ElliPro. Here, linear epitope sequences are

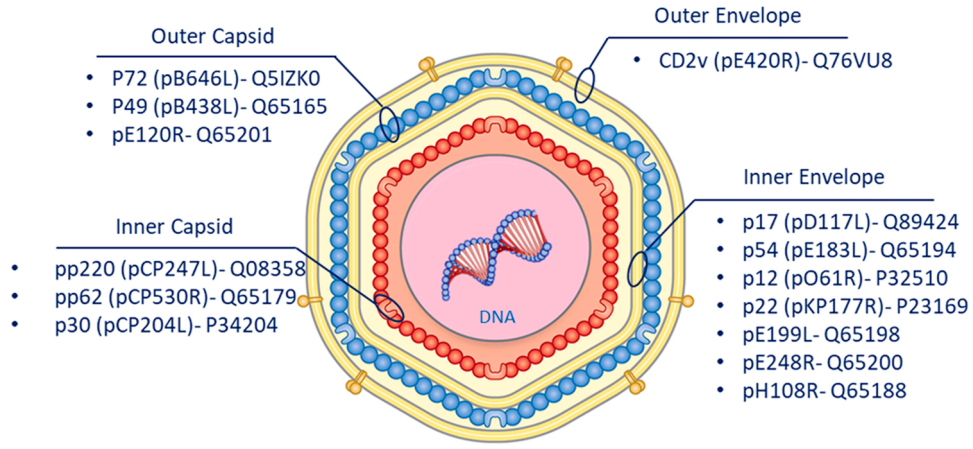

Fig. 1. African Swine Fever Virus (ASFV) particle and the selected structural protein. Major immunogenic proteins holding promising diagnostic potential are depicted in their viral particle localization. Each of the selected structural proteins is provided with its name and, in brackets, when available the alternative name. UniProt KnowledgeBase entry identifier is provided for each viral protein following the hyphen.

computed according to the proteins 3D structure. Briefly, the protein shape is approximated to a number of ellipsoids, enabling the labelling of the protruding residues and the calculation of the protrusion index (PI) for each protein residues based on the amino acid localization in relation to the ellipsoids; thus higher (PI) is indicative of a higher exposure and antibody accessibility (Ponomarenko et al., 2008).

2.4. Conformational epitopes prediction

ElliPro discontinuous epitopes are defined according to the PIs and clustered based on the distance between residue's centers of mass. In the present study, sequences with a minimum score of 0.5 were considered as a potential target of antibodies (Ponomarenko et al., 2008). Discontinuous B-cell epitope prediction by DiscoTope is accomplished on the protein three-dimensional structures by calculation of the surface accessibility, epitope propensity score and by considering spatial proximity and contact number among protein residues. Altogether, this yielded a DiscoTope score whose threshold was set to −3.5 ensuring the specificity of the method above 0.75 and sensitivity over 0.47 (Kringelum et al., 2012).

2.5. Epitopes quality assessment and structural mapping

The list of peptide epitopes resulting from the above calculations was further quality-checked and filtered, avoiding single amino acids epitopes and the inclusion of cross-reactive epitopes against the most common pathogens as of the blast analysis (https://blast.ncbi.nlm.nih.gov/Blast.cgi) of the selected protein sequences.

Epitopes mapping onto the PDB structural models relative to each of the selected viral proteins was performed using PyMOL tool and using a color code enabling the discrimation between the different bioinformatic tools and algorithms employed for the conformational epitopes prediction.

3. Results

The proteins selected in the present study are the structural entries with the highest likelihood of being targeted by direct diagnosis. Fig. 1 depicts the proteins of choice over the different layers of the viral particles, to enhance the detectability of the viral infection in the various stages of the infective process.

Among the selected proteins, only one entry (*i.e.* polyprotein 62, UniProtKB ID: Q65179) is provided with a well-defined 3D structure available in the PDB data repository. This raised the need to run independent *in-silico* predictions for the attribution of the protein sequences lacking an experimentally validated structure to the most suitable 3D model comprised in the PDB database. Details on the most suitable PDB structures, as attributed by SWISSMODEL tool, along with further information on the matching regions and quality is provided in Table 1.

Once assessed the viral structural protein of major interest and their spatial folding, principal immunogenic domains capable of eliciting antibody production have been predicted by a variety of immunoinformatic approaches specific for both linear and discontinuous epitopes. Prediction of linear B-epitopes has been accomplished by two independent algorithms (*i.e.* Bepipred and Ellipro) accounting for diverse epitope features, as detailed in the materials and methods above. Here, all the viral structural proteins showed at least one linear immunogenic sequence. However, most of the tested proteins scored a series of immunogenic sequences of variable lengths and only a handful of epitopes have shown a sequence length below 10 aminoacids. Importantly, several immunogenic sequences were labelled as B-epitopes by both independent computing approaches, supporting the epitopes prediction accuracy; whereas the other epitopes considered have been exclusively predicted by one of the two algorithms employed in linear B-epitopes prediction. The list of the linear B-epitopes as independently predicted by both Bepipred and Ellipro algorithms is provided in a table format as Supplementary Material S1.

Table 1
Structural folding prediction of the selected viral protein. The table summarizes the tridimensional folding prediction as of the available PDB structures. Details on the matching regions, protein sequence coverage and the matching similarity percentage with other known tridimensional structures are provided for each of the investigated proteins.

		SWISS-MODEL			
UniProtKB ID	PDB structure available	Code	Sequence region	Coverage %	Similarity %
Q5IZK0	NO	6L2T	1–646	100	97,37
Q76VU8	NO	1HNF	16–194	40	21,34
Q65179	YES	7BQ9	2–152	0,3	100
P23169	NO	2N28	6–36	0,18	25,81
Q65200	NO	6BM8	191–227	0,15	25
Q65165	NO	1SDI	298–349	0,12	23,08
Q65194	NO	7KDP	24–79	0,27	22
P32510	NO	7FFF	32–56	0,41	20
Q65188	NO	5FU3	74–98	0,23	20
Q65201	NO	2KVS	6–38	0,28	18,18
P34204	NO	6HSF	68–175	0,51	17,14
Q08358	NO	5TVL	295–344	0,02	15,91
Q65198	NO	4NV6	138–180	0,22	11,63
Q89424	NO	6NK5	26–61	0,31	11,11

The strongest immunogenic potential, on a whole protein basis, is scored by protein entry ID Q08358 as describing the highest number and length of B-epitopes as predicted by Bepipred algorithm. On the other hand, Ellipro-based epitopes computing highlighted the protein with UniProtKB ID Q5IZK0 as the most immunogenic entry based on the number and length of the linear B-epitopes.

Regardless of the number of predicted epitopes, candidate targets are evenly mapped over the entire length of the immunogenic proteins, suggesting a good versatility of the predicted sequences (Supplementary Material S1).

Analogously to the linear epitopes, prediction of the discontinuous epitopes was performed by two independent computing approaches, namely DiscoTope and ElliPro. Here, the molecular models previously attributed by SWISSMODEL were employed as the input file. Conformational epitopes prediction was accomplished independently for each algorithm, on a protein basis, resulting in the labelling of several protein sequences, of variable lengths, as capable of eliciting antibody production. The full list of epitope sequences, as computed by the two algorithms is provided for each of the selected ASFV proteins in Supplementary Material S2. Despite the identification of linear epitopes, the prediction of conformational epitopes in the viral proteins Q65165, Q89424 and Q65198 through the DiscoTope algorithm lack statistically valuable scores. On the other hand, ElliPro missed structural epitopes prediction in one of the two PDB models (*i.e.* 2KVS) relative to the protein Q65201, at the filters and thresholds chosen.

Mapping the epitope portions onto each 3D model highlighted a plurality of epitopes adjacent to each other, defining consistent immunogenic domains within each protein structure (Figs. 2, 3 and 4). Interestingly, good concordance has been registered among the two prediction algorithms, supporting the accuracy and reliability of the prediction computed. Also, other immunogenic domains have been exclusively highlighted by each of the two algorithms; interestingly, these often fall adjacent to each other and/or flanking the consensus epitopes, suggesting the presence of hot spot targets valuable for the direct viral diagnosis.

To further support the quality of the predicted epitopes, selected protein sequences were subjected to BLAST analysis to avert cross-reactivity against other pathogens. Here, all the selected proteins were blindly attributed to specimens of the Asfariviridae family at high sequence coverage and homology and only small portions of a few proteins highlighted potential cross-reactivity with other viral proteins

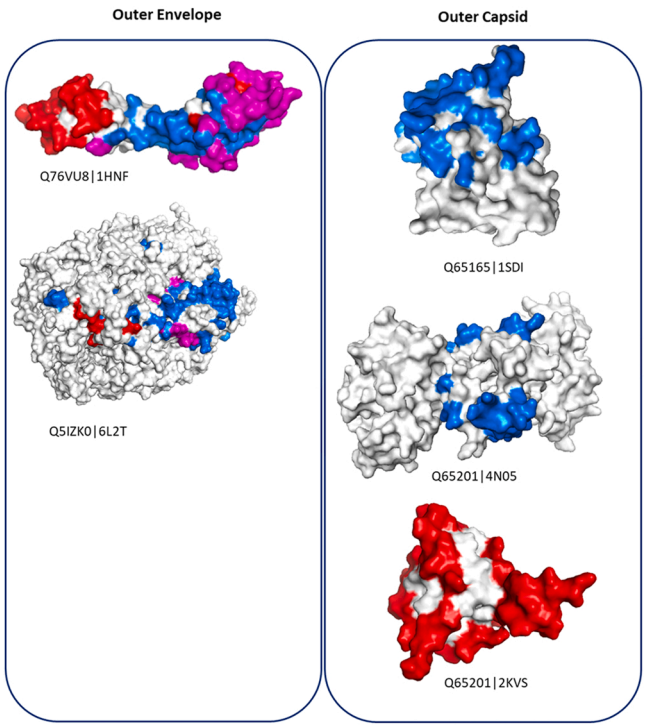

Fig. 2. Outer envelope and outer capsid protein conformational epitopes mapping. Conformational epitopes as predicted by the Ellipro and DiscoTope bioinformatic tools are mapped onto the attributed PDB models. Blue epitopes are the discontinuous epitope regions predicted by the ElliPro tool; Red moieties are the structural epitopes predicted by the DiscoTope algorithm; whilst consensus predictions are marked in magenta. UniProt KB identifiers are provided for each structural protein along with the 4-digits code relative to the attributed PDB structure.

and a handful of bacterial pathogens at low sequence coverage and accuracy as witnessed by the relative e-value. A comprehensive description of the BLAST analysis for each of the selected protein is provided in the Supplementary Material S3.

4. Discussion

African Swine Fever (ASF) is a recurrent problem severely affecting the global swine industry. Although non-zoonotic, the social and economic impact associated with this viral infection granted the inclusion of ASF in the list of the notifiable diseases by the World Organization for Animal Health (WOAH). Novel detection of ASFV in a new country or region results in the imposition of trade restrictions (Cwynar et al., 2019; Dixon et al., 2019) along with the activation of strict sanitary measures, including culling the infected herds as the only effective containment measures (Boklund et al., 2018) since no other effective therapeutic nor prophylactic intervention is so far available (Cwynar et al., 2019; King et al., 2011; Penrith et al., 2013). In addition, WOAH-recommended diagnostic strategies are rather time-consuming and less prone to in-field application, hindering the timely detection of ASF. This is particularly true in the developing countries and the remote rural areas that are poorly connected with the reference centers for ASF diagnosis and surveillance, making ASF control and eradication even harder. In this light, the optimization of rapid and in-field diagnosis has paramount importance for the containment of the viral diffusion and the reduction of animal losses. Diverse research groups across the globe are efforting in combining the discovery of promising biomarkers to frontiers technologies such as isothermal amplification, lateral flow chromatography and microfluidic chip finalized to the specific unbiased diagnosis of ASFV. As of today, sensitivity and/or versatility flaws have been registered while detecting the viral particle from the different sample kinds that may act as a vector of the pathogen. Thus, resulting in somehow biased diagnosis or underestimation of the ASFV diffusion in the animal herds (Cackett et al., 2020; Deutschmann et al., 2021; Dixon et al., 2019; DIxon et al., 2020). Recently, QDM based-ASFV immunosensor has been optimized for the quantitative detection of ASFV antibodies in serum with promising results (Zhu et al., 2022b). Authors expected exciting results in both qualitative and quantitative terms on a variety of sample kinds. Still, quantitative serological diagnosis accomplished by this innovative approach is less likely practicable on large scale in-farm screening of ASF, due to the difficulties encountered in the sampling from live animals either caged or in free-grazing breeding. Besides the practical aspects of the indirect diagnosis, it must be beard into account that ASFV has evolved with immune suppression mechanisms targeting both the host's innate response and the apoptotic processes that are commonly triggered by the host immune system in the attempt to reduce the viral diffusion (Wang et al., 2021). Moreover, ASFV hinders the adaptive immune response by inhibiting the expression of class I and II MHC molecules and the downstream activation of the cytotoxic T-lymphocytes. This results in a dramatic reduction of the overall immune response; thus, affecting the indirect diagnosis of ASF, especially in the early stages of the infection and/or outbreak onset (Liu et al., 2019).

Owing to the above reasons, this study focuses its rationale on the direct detection of the ASFV, thought as the most suitable solution for the rapid, in-field screening of ASF in a large-scale context. In this perspective, we considered the latest achievements by diverse research groups on the optimization of rapid direct screening tests. Altogether, a variety of diagnostic methodologies have been so far optimized, leading to the direct detection of the ASFV with impressive analytical performances (Carlson et al., 2018; Sastre et al., 2016; Ye et al., 2019; Zhu et al., 2022b). Nevertheless, each of the proposed tests suffers from specific limitations ranging from sensitivity, specificity, and diagnostic potential from different sample kinds (Deutschmann et al., 2021; Zhu et al., 2022b). Notably, all the proposed innovations rely on the targeting of a single immunogenic domain. In these author's view, the simultaneous targeting of an array of immunogenic proteins would greatly improve the diagnostic potential and accuracy. Here, the most promising immunogenic targets, from the studies available in the literature, were selected along with other viral structural proteins. The list of the selected proteins is such that structural representatives from all viral layers are considered. Enabling the detection of the different layers of the viral particle would benefit the diagnostic potential, as viral particles from all stages of the infective process can be virtually detected, promoting the direct diagnosis of ASF from the early stage of infection and/or ASFV detection from diverse biological samples including feces, saliva and animal carcasses where the viral particle can be found in a structurally damaged form. Altogether, this approach improves the ASF underestimation by considering the viral particles otherwise undetected by the single-target tests looking at the outer layer. Besides targeting multiple structural proteins, a plurality of epitopes from each of the selected proteins would further improve the screening capabilities of the test. The epitopes atlas provided in this study considers both linear and conformational epitopes capable of being recognized by antibodies, although other probes and/or chemicals might be employed for the detection of the ASFV target at a comparable specificity (Tans et al., 2020). Implementing both linear and conformational epitopes in a single test run widens the probability of catching the target of interest and, analogously to employing multiple immunogenic proteins, a linear epitope can provide a valuable diagnosis when a conformational epitope of the same antigen fails, and *vice versa*. This would overcome the issue of the ASF underestimation by false negative testing.

The study of the conformational epitopes *in silico* unavoidably requires *a priori* knowledge of the structural folding of the protein of interest. To date, the 3D structure of only one of the selected viral proteins has been elucidated and experimentally validated. This required the previous assessment of the proteins tridimensional structures to feed the

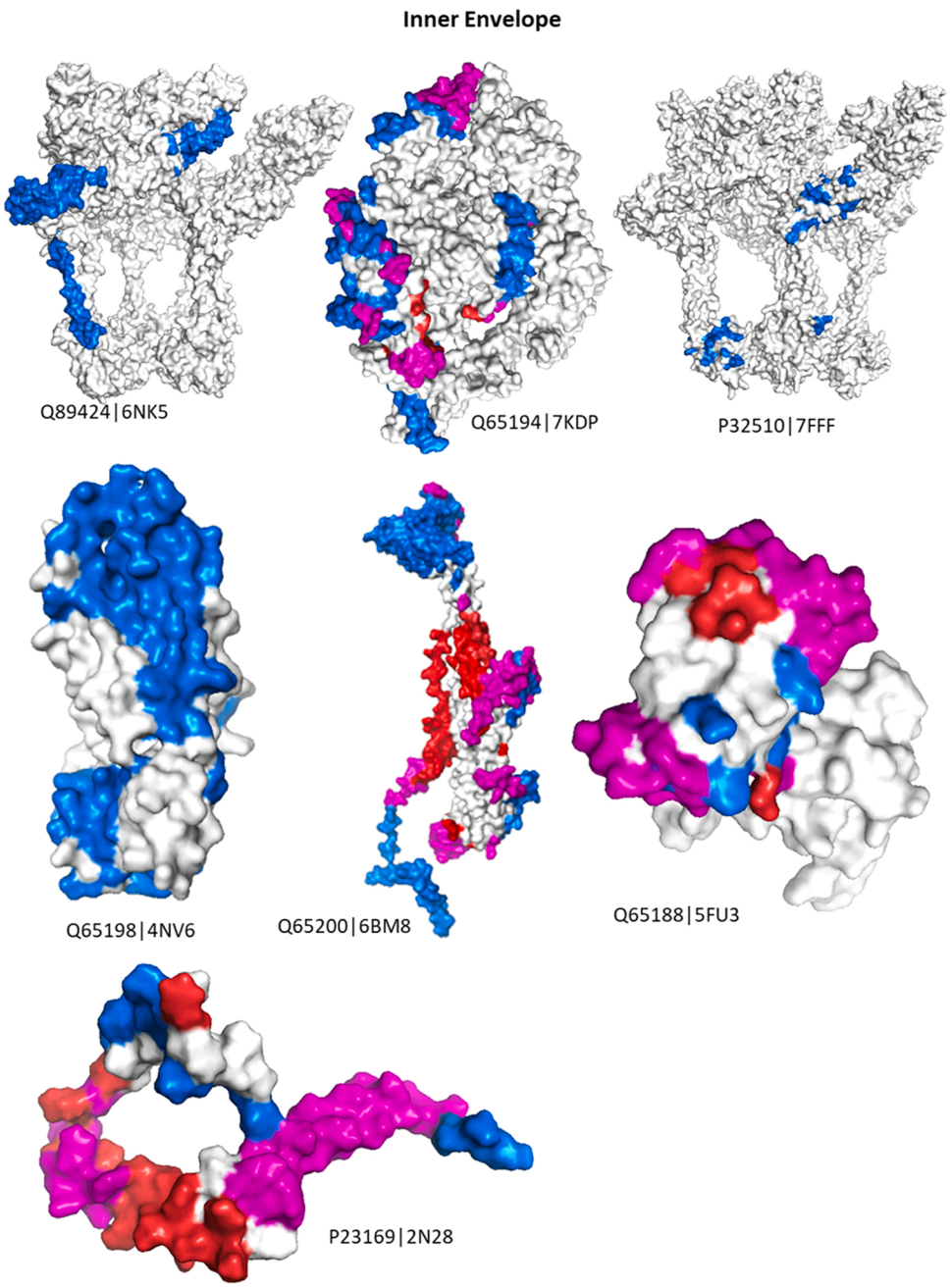

Fig. 3. Inner envelope protein conformational epitopes mapping. Conformational epitopes as predicted by the Ellipro and DiscoTope bioinformatic tools are mapped onto the attributed PDB models. Blue epitopes are the discontinuous epitope regions predicted by the ElliPro tool; Red moieties are the structural epitopes predicted by the DiscoTope algorithm; whilst consensus predictions are marked in magenta. UniProt KB identifiers are provided for each structural protein along with the 4-digits code relative to the attributed PDB structure.

algorithm predicting the conformational epitopes. As in all structural computing, the prediction of molecular folding was only possible through a portion of the whole sequence. In this view, conformational epitopes can be subclustered into two categories depending on their location in the protein sequence: (I) epitopes falling within the region employed for the attribution of the tridimensional folding as of the PDB databank and (II) the epitopes falling outside the above "matching region". Conformational epitopes of the I-type are more robust and accurate since their effective tridimensional folding is more closely represented by the PDB-available structure. Nevertheless, being a descriptive study providing the highest possible number of epitopes for the rapid screening of ASF, also the conformational epitopes of the II-type were considered and mapped in the relative protein model. In this light, the contribution of the II-type epitopes can be limited to supporting and/or reinforcing the annealing of the I-type epitopes; whereas care should be taken in posing diagnosis based on the sole targeting of the II-type epitopes.

BLAST analysis of the selected protein sequence reveal high predictive value for the direct detection of ASFV since all the protein entries are specifically attributed to the diverse specimens of the Asfariviridae family. Only a handful of protein portions scored a weak cross-reactive potential against other viruses and a very few bacterial pathogens. Here, the parameters employed as estimative of the cross-reactivity are rather weak since only very little portion, often even not considered as epitopes, are sherd with other microorganism at low homology percentage and coverage. In this light, optimization of the stringency condition while annealing the multiepitope would easily overcome the lack of specificity of the few shared protein portions.

Informatic-based computing of the T-epitopes is omitted as these are less likely suitable in the diagnostic settings, leaving its possible application to prophylactic purposes. On the other hand, besides having a great value in the direct diagnosis, prediction of the B-epitopes would be

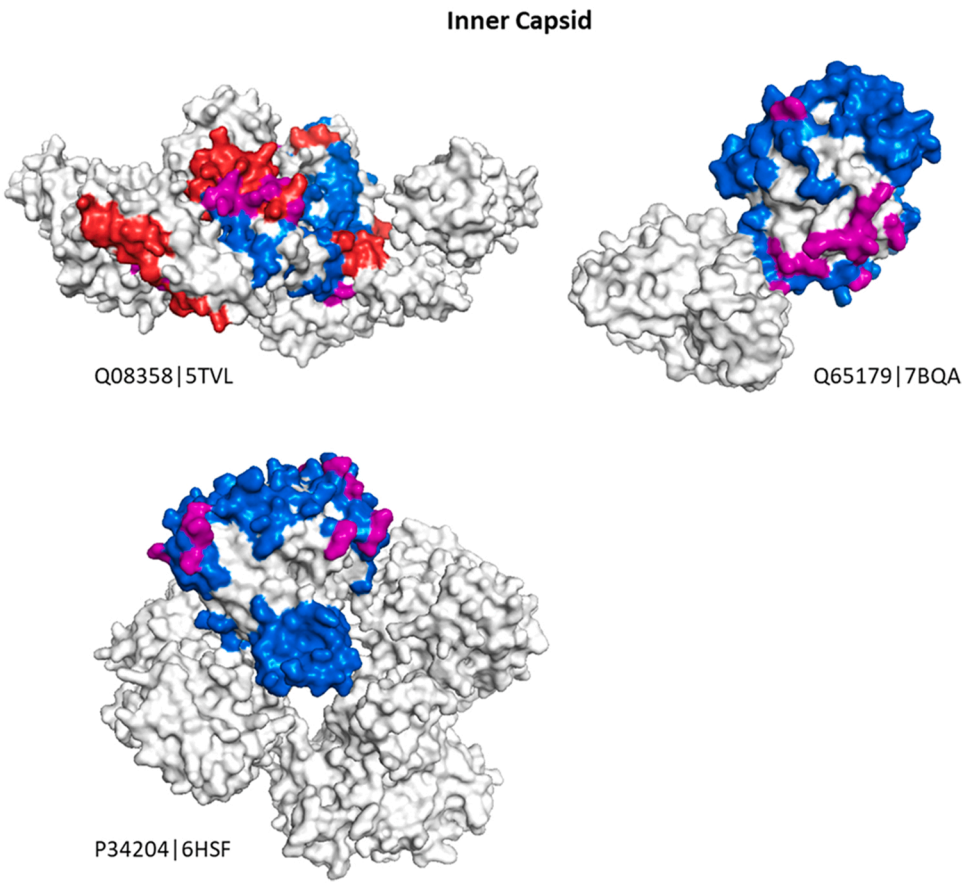

Fig. 4. Inner capsid protein conformational epitopes mapping. Conformational epitopes as predicted by the Ellipro and DiscoTope bioinformatic tools are mapped onto the attributed PDB models. Blue epitopes are the discontinuous epitope regions predicted by the ElliPro tool; Red moieties are the structural epitopes predicted by the DiscoTope algorithm; whilst consensus predictions are marked in magenta. UniProt KB identifiers are provided for each structural protein along with the 4-digits code relative to the attributed PDB structure.

of interest for the design of third tests aimed at defining the quantitative aspects of the diagnosis (*e.g.* assessing the effectiveness of prophylactic measures, viral titers etc), other than the rapid in-field diagnosis as of above.

5. Conclusion

The present study takes into account the warning problem of the ASF recurrence and underlines how the efforts in the development of innovative diagnostic tests against ASFV cannot succeed until targeting simultaneously a plurality of biomarkers that synergistically and supportively cooperate for the early diagnosis of ASF in a rapid and unbiased fashion. To follow up this new suggestive route, it provides the epitope atlas of the most promising structural protein of ASFV, and both linear and conformational epitopes are computed. It is worthy of note that provided epitopes are the results of informatic algorithm computations and, although strict filters, thresholds and statistical evaluations have been performed for the sorting of the most suitable sequences, preliminary *in vitro* tests are suggested and desirable to better define the diagnostic potential of each sequence. Altogether, this study provides the basic knowledge enabling the development of next-generation diagnostics. From this perspective, further studies will rely on this data for the effective implementation in automated screening programs such as machine learning approaches and the large-scale personalized medicine in the veterinary field.

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Declarations of interest

None.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.vetimm.2023.110548.

References

Alejo, A., Matamoros, T., Guerra, M., Andrés, G., 2018. A proteomic atlas of the African swine fever virus particle. J. Virol. 92. https://doi.org/10.1128/JVI.01293-18.

Alonso, C., Borca, M., Dixon, L., Revilla, Y., Rodriguez, F., Escribano, J.M., 2018. ICTV virus taxonomy profile: Asfarviridae. J. Gen. Virol. 99, 613–614. https://doi.org/10.1099/JGV.0.001049/CITE/REFWORKS.

Arias, M., Sánchez-Vizcaíno, J.M., Morilla, A., Yoon, K.J., Zimmerman, J.J., 2002. African swine fever. Trends Emerg. Viral Infect. Swine 119–124.

Bateman, A., Martin, M.J., Orchard, S., Magrane, M., Agivetova, R., Ahmad, S., Alpi, E., Bowler-Barnett, E.H., Britto, R., Bursteinas, B., Bye-A-Jee, H., Coetzee, R., Cukura, A., da Silva, A., Denny, P., Dogan, T., Ebenezer, T.G., Fan, J., Castro, L.G., Garmiri, P., Georghiou, G., Gonzales, L., Hatton-Ellis, E., Hussein, A., Ignatchenko, A., Insana, G., Ishtiaq, R., Jokinen, P., Joshi, V., Jyothi, D., Lock, A., Lopez, R., Luciani, A., Luo, J., Lussi, Y., MacDougall, A., Madeira, F., Mahmoudy, M., Menchi, M., Mishra, A., Moulang, K., Nightingale, A., Oliveira, C.S., Pundir, S., Qi, G., Raj, S., Rice, D., Lopez, M.R., Saidi, R., Sampson, J., Sawford, T., Speretta, E., Turner, E., Tyagi, N., Vasudev, P., Volynkin, V., Warner, K., Watkins, X., Zaru, R., Zellner, H., Bridge, A., Poux, S., Redaschi, N., Aimo, L., Argoud-Puy, G., Auchincloss, A., Axelsen, K., Bansal, P., Baratin, D., Blatter, M.C., Bolleman, J., Boutet, E., Breuza, L., Casals-Casas, C., de Castro, E., Echioukh, K.C., Coudert, E., Cuche, B., Doche, M., Dornevil, D., Estreicher, A., Famiglietti, M.L., Feuermann, M., Gasteiger, E., Gehant, S., Gerritsen, V., Gos, A., Gruaz-Gumowski, N., Hinz, U., Hulo, C., Hyka-Nouspikel, N., Jungo, F., Keller, G., Kerhornou, A., Lara, V., Le Mercier, P., Lieberherr, D., Lombardot, T., Martin, X., Masson, P., Morgat, A., Neto, T.B., Paesano, S., Pedruzzi, I., Pilbout, S., Pourcel, L., Pozzato, M., Pruess, M., Rivoire, C., Sigrist, C., Sonesson, K., Stutz, A., Sundaram, S., Tognolli, M., Verbregue, L., Wu, C.H., Arighi, C.N., Arminski, L., Chen, C., Chen, Y., Garavelli, J.S., Huang, H., Laiho, K., McGarvey, P., Natale, D.A., Ross, K., Vinayaka, C.R., Wang, Q.,

Wang, Y., Yeh, L.S., Zhang, J., 2021. UniProt: the universal protein knowledgebase in 2021. Nucleic Acids Res. 49, D480–D489. https://doi.org/10.1093/NAR/GKAA1100.

Boklund, A., Cay, B., Depner, K., Földi, Z., Guberti, V., Masiulis, M., Miteva, A., More, S., Olsevskis, E., Satr an, P., Spiridon, M., Stahl, K., Thulke, H.-H., Viltrop, A., Wozniakowski, G., Broglia, A., Cortinas Abrahantes, J., Dhollander, S., Gogin, A., Verdonck, F., Amato, L., Papanikolaou, A., Gort azar, C., Bocian, Ł., Chesnoiu, T., andor Csanyi, S., Feliziani, F., sauskien, Hoffman, G., Jaarma, A., art, Judickas, M., okay Katalin, J., Keuling, O., Kocsis, M., Korytarova, D., Mihaita, M., Neil, A., Pe drakowska, K., Pittiglio, C., Podgorski, T., Ser zants, artin ß, Sautoer-Louis, M., Urbelionyt, C., Velizar, J., Vicente Banos, B., Wallo, J., Zihmanis, I, R., 2018. Epidemiological analyses of African swine fever in the European Union (November 2017 until November 2018). EFSA J. 16, e05494 https://doi.org/10.2903/J.EFSA.2018.5494.

Cackett, G., Sýkora, M., Werner, F., 2020. Transcriptome view of a killer: African swine fever virus. Biochem. Soc. Trans. 48, 1569–1581. https://doi.org/10.1042/BST20191108.

Carlson, J., Zani, L., Schwaiger, T., Nurmoja, I., Viltrop, A., Vilem, A., Beer, M., Blome, S., 2018. Simplifying sampling for African swine fever surveillance: assessment of antibody and pathogen detection from blood swabs. Transbound. Emerg. Dis. 65, e165–e172. https://doi.org/10.1111/TBED.12706.

Cwynar, P., Stojkov, J., Wlazlak, K., 2019. African swine fever status in Europe. Viruses 11. https://doi.org/10.3390/V11040310.

Deutschmann, P., Pikalo, J., Beer, M., Blome, S., 2021. Lateral flow assays for the detection of African swine fever virus antigen are not fit for field diagnosis of wild boar carcasses. Transbound. Emerg. Dis. https://doi.org/10.1111/TBED.14248.

Dixon, L.K., Sun, H., Roberts, H., 2019. African swine fever. Antivir. Res 165, 34–41. https://doi.org/10.1016/J.ANTIVIRAL.2019.02.018.

DIxon, L.K., Stahl, K., Jori, F., Vial, L., Pfeiffer, Di.U., 2020. Afr. Swine Fever Epidemiol. Control 8, 221–246. https://doi.org/10.1146/ANNUREV-ANIMAL-021419-083741.

Gallardo, C., Nieto, R., Soler, A., Pelayo, V., Fernández-Pinero, J., Markowska-Daniel, I., Pridotkas, G., Nurmoja, I., Granta, R., Simón, A., Pérez, C., Martín, E., Fernández-Pacheco, P., Arias, M., 2015. Assessment of African swine fever diagnostic techniques as a response to the epidemic outbreaks in Eastern European Union Countries: how to improve surveillance and control programs. J. Clin. Microbiol. 53, 2555–2565. https://doi.org/10.1128/JCM.00857-15.

Gao, Y., Meng, X.Y., Zhang, H., Luo, Y., Sun, Y., Li, Y., Abid, M., Qiu, H.J., 2018. Cross-priming amplification combined with immunochromatographic strip for rapid on-site detection of African swine fever virus. Sens. Actuators B Chem. 274, 304–309. https://doi.org/10.1016/J.SNB.2018.07.164.

Jespersen, M.C., Peters, B., Nielsen, M., Marcatili, P., 2017. BepiPred-2.0: improving sequence-based B-cell epitope prediction using conformational epitopes. Nucleic Acids Res 45, W24–W29. https://doi.org/10.1093/NAR/GKX346.

King, K., Chapman, D., Argilaguet, J.M., Fishbourne, E., Hutet, E., Cariolet, R., Hutchings, G., Oura, C.A.L., Netherton, C.L., Moffat, K., Taylor, G., Le Potier, M.F., Dixon, L.K., Takamatsu, H.H., 2011. Protection of European domestic pigs from virulent African isolates of African swine fever virus by experimental immunisation. Vaccine 29, 4593. https://doi.org/10.1016/J.VACCINE.2011.04.052.

Kringelum, J.V., Lundegaard, C., Lund, O., Nielsen, M., 2012. Reliable B cell epitope predictions: impacts of method development and improved benchmarking. PLoS Comput. Biol. 8. https://doi.org/10.1371/JOURNAL.PCBI.1002829.

Liu, Xi, Zheng, H., Lu, R., Huang, H., Zhu, H., Yin, C., Mo, Y., Wu, J., Liu, Xuwen, Deng, M., Li, D., Cheng, B., Wu, F., Liang, Y., Guo, H., Song, H., Su, Z., 2019. Intervening effects of total alkaloids of corydalis saxicola bunting on rats with antibiotic-induced gut microbiota dysbiosis based on 16s rrna gene sequencing and untargeted metabolomics analyses. Front. Microbiol. 10, 1151. https://doi.org/10.3389/FMICB.2019.01151/BIBTEX.

Mazur-Panasiuk, N., Żmudzki, J., Woźniakowski, G., 2019. African swine fever virus – persistence in different environmental conditions and the possibility of its indirect transmission. J. Vet. Res. 63, 303. https://doi.org/10.2478/JVETRES-2019-0058.

Oura, C.A.L., Edwards, L., Batten, C.A., 2013. Virological diagnosis of African swine fever–comparative study of available tests. Virus Res 173, 150–158. https://doi.org/10.1016/J.VIRUSRES.2012.10.022.

Penrith, M.L., Vosloo, W., Jori, F., Bastos, A.D.S., 2013. African swine fever virus eradication in Africa. Virus Res 173, 228–246. https://doi.org/10.1016/J.VIRUSRES.2012.10.011.

Ponomarenko, J., Bui, H.H., Li, W., Fusseder, N., Bourne, P.E., Sette, A., Peters, B., 2008. ElliPro: a new structure-based tool for the prediction of antibody epitopes. BMC Bioinforma. 9. https://doi.org/10.1186/1471-2105-9-514.

Sánchez-Vizcaíno, J.M., Mur, L., Martínez-López, B., 2012. African swine fever: an epidemiological update. Transbound. Emerg. Dis. 59 (Suppl 1), 27–35. https://doi.org/10.1111/J.1865-1682.2011.01293.X.

Sastre, P., Pérez, T., Costa, S., Yang, X., Räber, A., Blome, S., Goller, K.V., Gallardo, C., Tapia, I., García, J., Sanz, A., Rueda, P., 2016. Development of a duplex lateral flow assay for simultaneous detection of antibodies against African and Classical swine fever viruses. J. Vet. Diagn. Invest. 28, 543–549. https://doi.org/10.1177/1040638716654942.

Scientific Opinion on African swine fever | EFSA [WWW Document], n.d. URL https://www.efsa.europa.eu/it/efsajournal/pub/3628 (accessed 5.9.22).

Tans, R., van Rijswijck, D.M.H., Davidson, A., Hannam, R., Ricketts, B., Tack, C.J., Wessels, H.J.C.T., Gloerich, J., van Gool, A.J., 2020. Affimers as an alternative to antibodies for protein biomarker enrichment. Protein Expr. Purif. 174, 105677 https://doi.org/10.1016/J.PEP.2020.105677.

Trotta, A., Marinaro, M., Cavalli, A., Cordisco, M., Piperis, A., Buonavoglia, C., Corrente, M., 2022. African swine fever–how to unravel fake news in veterinary medicine. Anim 2022 Vol. 12. https://doi.org/10.3390/ANI12050656.

Vita, R., Mahajan, S., Overton, J.A., Dhanda, S.K., Martini, S., Cantrell, J.R., Wheeler, D. K., Sette, A., Peters, B., 2019. The immune epitope database (IEDB): 2018 update. Nucleic Acids Res 47, D339–D343. https://doi.org/10.1093/NAR/GKY1006.

Wang, A., Jia, R., Liu, Y., Zhou, J., Qi, Y., Chen, Y., Liu, D., Zhao, J., Shi, H., Zhang, J., Zhang, G., 2020. Development of a novel quantitative real-time PCR assay with lyophilized powder reagent to detect African swine fever virus in blood samples of domestic pigs in China. Transbound. Emerg. Dis. 67, 284–297. https://doi.org/10.1111/TBED.13350.

Wang, Y., Kang, W., Yang, W., Zhang, J., Li, D., Zheng, H., 2021. Structure of African swine fever virus and associated molecular mechanisms underlying infection and immunosuppression: a review. Front. Immunol. 12. https://doi.org/10.3389/FIMMU.2021.715582.

Waterhouse, A., Bertoni, M., Bienert, S., Studer, G., Tauriello, G., Gumienny, R., Heer, F. T., De Beer, T.A.P., Rempfer, C., Bordoli, L., Lepore, R., Schwede, T., 2018. SWISS-MODEL: homology modelling of protein structures and complexes. Nucleic Acids Res 46, W296–W303. https://doi.org/10.1093/NAR/GKY427.

Ye, X., Li, L., Li, J., Wu, X., Fang, X., Kong, J., 2019. Microfluidic-CFPA chip for the point-of-care detection of African swine fever virus with a median time to threshold in about 10 min. ACS Sensors 4, 3066–3071. https://doi.org/10.1021/ACSSENSORS.9B01731/SUPPL_FILE/SE9B01731_SI_001.PDF.

Zardecki, C., Dutta, S., Goodsell, D.S., Lowe, R., Voigt, M., Burley, S.K., 2022. PDB-101: Educational resources supporting molecular explorations through biology and medicine. Protein Sci. 31, 129–140. https://doi.org/10.1002/PRO.4200.

Zhai, Y., Ma, P., Fu, X., Zhang, L., Cui, P., Li, H., Yan, W., Wang, H., Yang, X., 2020. A recombinase polymerase amplification combined with lateral flow dipstick for rapid and specific detection of African swine fever virus. J. Virol. Methods 285, 113885. https://doi.org/10.1016/J.JVIROMET.2020.113885.

Zhu, W., Meng, K., Zhang, Y., Bu, Z., Zhao, D., Meng, G., 2022a. Lateral flow assay for the detection of African swine fever virus antibodies using gold nanoparticle-labeled acid-treated p72. Front. Chem. 9 https://doi.org/10.3389/FCHEM.2021.804981/FULL.

Zhu, W., Meng, K., Zhang, Y., Bu, Z., Zhao, D., Meng, G., 2022b. Lateral flow assay for the detection of African swine fever virus antibodies using gold nanoparticle-labeled acid-treated p72. Front. Chem. 9 (1164) https://doi.org/10.3389/FCHEM.2021.804981/BIBTEX.