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Porcine Serum, Trypsin, and Other Porcine-Derived Products: *Swine Viruses of Importation and Adventitious Concern*

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Abstract

The rapid and seemingly uncontrolled spread of African swine fever (ASF) throughout China and many of its neighboring countries within the last 19 months (August 2018–March 2020) has put the rest of the world on high alert. The geographic distribution of viruses of importation concern, like ASF virus (ASFV), can change very quickly, putting at risk conventional sources of porcine serum and other porcine-derived products used as ingredients in research, the manufacture of biologics, and other biomedical applications. This article reviews the 2019 information from the World Organization for Animal Health (OIE) regarding the presence or absence of eight viruses of importation concern in the swine populations of 30 countries from animal serum-producing regions of the world. Companies importing porcine raw materials for formulation into porcine products — and their customers — should be aware of the geographic location of swine diseases of importation concern. The article also identifies ten adventitious viruses of concern cited in United States Department of Agriculture (USDA) and European Union (EU) regulations that need to be tested for or eliminated through one or more barrier treatments when porcine ingredients are used in the manufacture of biologics.

1. Introduction

African swine fever (ASF) is currently the disease of greatest concern when importing swine products. Even though the disease was eradicated from Europe in the 1990s, it reentered eastern Europe in 2007 through the Caucasus and has since spread to several other European and former Soviet Union countries. The ASF virus (ASFV) situation in Europe is very dynamic, and the World Organization for Animal Health (OIE) website maintains up-to-date information on the spread of this disease throughout Europe, Africa, and Asia. For example, ASFV recently spread into China (August 2018) and has extended into neighboring regions (August 2018–March 2020).[1] This is very troubling to the swine industry and stakeholders worldwide. It is believed that ASFV will continue to spread through all of Asia and perhaps to other continents via contaminated pork products and animal feed, or simply by the presence of the virus on the shoes and clothing of people moving from one country to the next.

ASF is one of eight viral diseases of importation concern for swine-origin products, identified in United States Department of Agriculture (USDA)[2] and European Union (EU)[3] import regulations. An additional ten adventitious viruses are identified by USDA and EU regulations as potential contaminants in porcine ingredients, regardless of the country of origin. Companies involved in the collection, processing, sale, or purchase of porcine products should be familiar with the swine diseases of concern that can affect the safety and use of their products.

Porcine serum, like fetal bovine serum (FBS) and other animal sera, is used as a supplement in growth culture media for research, vaccine production, diagnostic tests, and other bioapplications. Porcine trypsin is a proteolytic enzyme extracted from swine pancreatic glands collected at slaughter. It is prepared as a powder or liquid solution for use as a reagent in cell culture applications and as a protein processing reagent. Other related products used in biomedical research, manufacturing, and technology are porcine albumin, IgG, and heme, as well as various other porcine tissues and glands.

This article will identify the porcine viruses cited as concerns in USDA and EU regulations. A comparison will be made of the animal health status of 30 countries in serum-producing regions of the world, based on 2019 information from the OIE.[4]

2. OIE Standards

The OIE has listed diseases of concern for the import/export of animals and their byproducts, as well as the trade standards relating to each disease. The OIE has also established two initiatives for recognizing the animal health status of its 182 member countries. One is called the "official disease status" and includes a comprehensive evaluation and recognition of the disease status of a country, as determined by the OIE. This initiative has been implemented for seven livestock diseases including four that affect swine: classical swine fever (CSF), foot and mouth disease (FMD), peste des petits ruminants (PPR), and rinderpest (RP).[5] The second initiative for the recognition of disease-free status is called the "self-declared disease status.[6] This is based on a set of criteria established by the OIE where a member country can declare or affirm themselves to be free of a specific animal disease.

The purpose of OIE trade standards is to assure the absence of disease-causing agents in imported animal products by prohibiting importation or using other measures such as safety testing or barrier treatments (*e.g.*, gamma irradiation or heat treatment). Each country develops their own import regulations, hopefully based on OIE standards and the presence or absence of the diseases of concern in the livestock populations of their own country and also their trading partners.

3. USDA- and EU-Authorized Countries of Origin

Since the two largest global markets for animal-derived products are the USA and Europe, the import requirements from USDA and the European Commission (EC), to a great extent, have become the veterinary control standards for the serum industry. Approved countries of origin for importing swine serum are closely related to the approved countries of origin for importing fresh pork. Countries authorized to export fresh pork to the USA and the EU can be considered safe sources of porcine serum and other porcine tissues.

The USDA allows the importation of fresh pork from approved slaughterhouses in countries or zones listed in "eligible countries and products."[7] As of March 2020, approved slaughterhouses from 15 countries were authorized to export fresh pork to the USA: **Brazil (from the state of Santa Catarina), Canada, Chile, Denmark, England, Finland, France, Hungary, Ireland, Italy, Mexico, Netherlands, Northern Ireland, Poland, and Spain.** In order to import porcine serum, trypsin, or other porcine products into the USA from these countries, the importer must apply for an import permit. USDA may also consider approving import applications for porcine products from other countries if the products are heat treated or safety tested at USDA's Foreign Animal Disease Diagnostic Lab (FADDL).[8]

The EU also publishes a list of countries allowed to export fresh pork to the EU as part of EU Regulation No. 206/2010 known as "the third country list." As of July 2019, five countries appear on this list as eligible to export fresh pork to the EU (**Australia, Canada, Chile, New Zealand, and USA**).[9]

USDA and EU requirements for importing porcine products are very similar, except for the criteria used to recognize a trading-partner country to be free of a particular disease. The EU recognizes the official animal health status of their trading-partner countries, as determined by the OIE. On the other hand, the USDA does not use the OIE official animal health status of member countries. Rather, the USDA considers diseases like FMD, CSF, and swine vesicular disease (SVD) "to exist in all regions of the world except those declared free of the disease by USDA Animal and Plant Health Inspection Service (APHIS)."[2] A list of countries and regions that APHIS has declared free of these and other diseases can be found on USDA's website: Animal Health Status of Regions.[10]

4. Swine Viruses of Importation Concern

Eight diseases of importation concern have been identified by USDA[2] and EU[3] regulatory authorities for the importation of porcine products. Two of the eight diseases are caused by swine fever viruses, three are caused by vesicular disease viruses, and three are caused by viruses that are absent throughout the Americas, Europe, and Oceania.

Table 1 (on the next page) has been prepared using 2019 OIE data to compare the presence or absence of these eight viruses of importation concern in 30 countries from three regions of the world where animal serum is currently collected (Americas, Europe, and Asia/Oceania). Sixteen of the 30 countries compared report the absence of all eight viruses of importation concern, while the remaining 14 countries report the presence of one, two, or six viruses of importation concern.

4.1 Swine Fever Viruses

ASFV and CSF virus (CSFV) both cause similar and often indistinguishable clinical manifestations in swine herds. In the acute form, the manifestations include high fever and high mortality rates of 90–100%. Neither of these viruses affect humans.[11,12]

African swine fever virus had been eradicated from Europe in 1995, but ASFV re-entered Europe in 2007 through Georgia and spread to Russia. Since then, ASF has remained out of control, due to its presence in wild swine, and has affected 19 European countries.[13] China reported its first case of ASF in September of 2018. The rapid spread of the disease throughout China and 12 of its neighboring Asian countries has caused great concern, since the disease has spread much more quickly than anticipated.[14] It is believed that the disease will continue to spread throughout Asia and to other countries and continents by the movement of contaminated meat products, oil seed feed products[15], and virus contamination on the shoes and clothing of people traveling from one country to another. East Timor, one of the latest countries to acquire ASF in Southeast Asia, puts the disease only 404 miles from northern Australia, which has an estimated population of 24 million wild swine.[16] The Asian countries now reporting the disease have two thirds of the world's population of swine (522 of the 770 million estimated global population.[17]

No country, island, or continent is exempt from importing ASF. For example, in March of 2019, US Customs and Border Protection agents in New Jersey reported the seizure of one million pounds of illegally smuggled Chinese pork, the biggest seizure of agriculture products in the history of the USA.[18]

Classical swine fever virus has, at one time or another, affected most countries throughout the world. However, successful eradication programs and cooperation between

TABLE 1. Comparison of the swine health status of 30 countries for viruses of importation concern (2019). Sources: USDA[2], EU[3], and OIE[4].

	African Swine Fever	Classical Swine Fever	Foot and Mouth Disease	Swine Vesicular Disease *2014 OIE data*[4]	Vesicular Stomatitis *2014 OIE data*[4]	Rinderpest	Peste des Petits Ruminants	Rift Valley Fever	Total Viruses of Importation Concern
AMERICAS									
Argentina	—	1999	2006	—	1986	—	—	—	0
Brazil	1981	+	2006	—	+	1921	—	—	2
Canada	—	1963	1952	—	1949	—	—	—	0
Chile	—	1996	1987	—	—	—	—	—	0
Colombia	—	+	2018	—	+	—	—	—	2
Costa Rica	—	1997	—	—	+	—	—	—	1
Guatemala	—	2013	—	—	+	—	—	—	1
Mexico	—	2009	1954	—	+	—	—	—	1
Nicaragua	—	2008	—	—	+	—	—	—	1
Panama	—	1961	—	—	+	—	—	—	1
USA	—	1976	1929	—	+	—	—	—	1
Uruguay	—	1991	2001	—	—	—	—	—	0
EUROPE	*								
Denmark	—	1933	1983	—	—	1782	—	—	0
Finland	—	1917	1959	—	—	1877	—	—	0
France	1974	2002	2001	1983	—	1870	—	—	0
Germany	—	2006	1988	1985	—	1870	—	—	0
Hungary	+	1993	1973	—	—	1881	—	—	1
Ireland	—	1958	2001	—	—	1866	—	—	0
Italy	+	2003	1993	+	—	1947	—	—	2
Lithuania	+	2011	1982	—	—	-	—	—	1
Netherlands	1986	1998	2001	1994	—	1869	—	—	0
Norway	—	1963	1952	—	—	—	—	—	0
Poland	+	1994	1971	1972	—	1921	—	—	1
Spain	1994	2002	1986	1993	—	—	—	—	0
Sweden	—	1944	1966	—	—	1700	—	—	0
United Kingdom	—	2000	2007	1982	—	1877	—	—	0
ASIA/OCEANIA	**								
Australia	—	1962	1871	—	—	1923	—	—	0
China	+	+	+	No info	—	1955	+	No info	6
Japan	—	+	2010	1975	—	1922	—	—	1
New Zealand	—	1953	—	—	—	-	—	—	0

+ = Disease present — = Disease never reported **Year** = Year disease last reported **No info** = Disease considered present

* European countries affected with ASF (2007–March 2020): Armenia, Azerbaijan, Belarus, Belgium, Bulgaria, Estonia, Georgia, Greece, Hungary, Italy, Latvia, Lithuania, Moldova, Poland, Romania, Russia, Serbia, Slovakia, and Ukraine.

** Asian countries affected with ASF (September 2018–March 2020): Cambodia, China, East Timor, Hong Kong, Indonesia, Laos, Mongolia, Myanmar, Papua New Guinea, North Korea, Philippines, South Korea, and Vietnam.

neighboring countries has liberated it from most countries in the Americas, Europe, and Oceania. Nevertheless, the disease continues to be endemic in several countries of South and Central America, parts of eastern Europe, as well as Asia and Africa.[19]

4.2 Vesicular Disease Viruses

FMD virus (FMDV), SVD virus (SVDV), and vesicular stomatitis virus (VSV) cause diseases with similar clinical manifestations, since all cause vesicular lesions, which can be found in the feet, mouth, snout, and teats of affected animals. Affected animals

normally recover to full health within a few weeks after the vesicular lesions heal. All three of these diseases can occasionally affect humans.

Foot and mouth disease virus is one of the most contagious animal viruses causing diseases of economic importance for cloven-hoofed animals, and has at one time or another affected all regions and most countries throughout the world, including the USA, Canada, Mexico, Australia, South America, and Europe. Today North, Central, and South America are recognized by the OIE to be free of FMD either with or without vaccination (with the exception of Venezuela). Europe and Oceania are also free of FMD without vaccination. Even though there are several other countries and zones considered by the OIE to be free of FMD either with or without vaccination, parts of Asia, Africa, and the Middle East continue to be endemic for FMD.[20]

Vesicular stomatitis virus is of concern to the European countries because the virus only exists in the Americas, and because VS's clinical presentation is identical to that of FMD. VS is spread by insects and is endemic in the tropical and semi-tropical areas of the Americas. Every few years, however, the VSV spreads into the adjacent more temperate areas of both North and South America[21], as it did in 2019, affecting eight western and midwestern states in the USA.[22]

Swine vesicular disease virus is of concern to veterinary authorities in both the Americas and Europe because SVD's clinical presentation is identical to that of FMD, and because the disease is regularly reported in Italy and occasionally in other European countries. The disease is also present in various parts of eastern Asia.[23]

4.3 Viruses Absent from the Americas, Europe, and Oceania

RP, PRR, and Rift Valley fever (RVF) are diseases of concern to Europe because they either currently occur in nearby countries or regions of the world, or present a risk of re-emerging due to the presence of live RVF virus (RVFV) samples stored in diagnostic and research laboratories. The USDA also lists these three viruses as biological agents which "have the potential to pose a severe threat to public health and safety, and to animal health or to animal products."[24] RVFV can cause severe disease in humans, while RP virus (RPV) and PRR virus (PRRV) do not.

Rinderpest virus, a virus of African origin, spread to many countries throughout the Old World (Africa, Asia, and Europe) during the 1700s through the early 1900s. Successful control and eradication programs allowed many countries to become free of the RP disease in the late 1800s. Control and eradication efforts intensified after 1945 with leadership from the Food and Agriculture Organization (FAO) of the United Nations, and in 2011, the FAO officially declared the disease eradicated in livestock throughout the entire world.[25] Nevertheless, the disease continues to be of regulatory concern, since outbreaks could reappear due to existing virus stocks, vaccines, and biological samples stored in several locations around the world.[26]

Peste des petits ruminants virus causes a disease similar to RP but affects primarily small ruminants as well as swine and a few other species. The disease is endemic in parts of Africa, the Middle East, as well as Central, Southern, and Southeast Asia.[27] In 2015, the FAO and OIE launched a global campaign to eradicate PPR by 2030.

Rift Valley fever virus is carried by mosquitoes and causes a disease affecting ruminants, rodents, and a few other species including humans. It is endemic in eastern and southern Africa and spreads into other areas of Africa and the Arabian Peninsula during heavy rainy seasons, every 5–25 years.[28]

5. Adventitious Swine Viruses

In addition to swine viruses of importation concern, USDA[29, 30] and EU[31] have specifically identified ten adventitious viruses that, regardless of the country of origin, may be present in porcine serum, trypsin, and other porcine tissues

Ten Adventitious Swine Viruses of Concern:

Bovine viral diarrhea virus (BVDV) is a member of the *Flaviviridae* family. BVDV is the most common contaminant of bovine serum and can also infect pigs, sheep, goats, and deer.

Porcine adenovirus (PAV), of the family *Adenoviridae*, causes gastrointestinal disease in young pigs and is thought to contribute to porcine respiratory diseases.

Porcine hemagglutinating encephalitis virus (PHEV) is a member of the *Coronaviridae* family that causes vomiting, constipation, and anorexia, and rapid death or chronic emaciation (vomiting and wasting) in young swine.

Porcine parvovirus (PPV), family *Parvoviridae*, is a common and important cause of infectious infertility, stillbirths, and embryonic death in large swine herds.

Reovirus, *Reoviridae* family, affects many animal species, causing diarrhea in piglets, as well as pneumonia, encephalitis, and reproductive failure in swine.

Rabies virus (RV) is member of the *Rhabdoviridae* family that causes a disease of public and veterinary health concern which can affect all mammals. Rabies has been eradicated from several countries in Europe and Oceania.

Transmissible gastroenteritis virus (TGEV) is a highly infectious member of the *Coronaviridae* family that causes disease in pigs, causing diarrhea and death in young piglets.

Porcine circovirus (PCV), family *Circoviridae*, affects mainly small piglets, causing poor growth rate, weight loss, severe thinning, weakness, and sudden death.

Pseudorabies virus (PRV) is a member of the *Herpesviridae* family that predominantly affects swine but can also affect many other mammal species.

Seneca Valley virus (SVV) causes vesicular-like lesions in swine, similar to lesions caused by two other members of the *Picornaviridae* family (FMDV and SVV). It should be noted that testing requirements for SVV were only recently announced in 2018 by the USDA Center for Veterinary Biologics (CVB) when the virus was found to contaminate porcine trypsin and porcine serum.[30]

collected for use in cell culture media and other applications. Trypsin, because of its proteolytic affect, is known to inactivate many viruses. However, three of the ten viruses are identified as possible contaminants of trypsin: porcine parvovirus (PPV), porcine coronavirus (PCV), and Seneca Valley virus (SVV). These three viruses are the smallest of the ten adventitious viruses. Of the three, only SVV would be expected to be inactivated to an appreciable extent by gamma irradiation at the doses normally applied (20–50 kGy).[32]

The 9 CFR 113 testing requirements of USDA for adventitious viruses are capable of detecting many, but not all of the viruses capable of contaminating swine products. In 2011, Marcus-Sekura *et al*. published a list of 52 swine viruses from various parts of the world that have been reported in scientific literature. Of the 52 viruses, 18 were not expected to be detected by 9 CFR 113 testing assays.[33] The USDA, the US Food and Drug Administration (FDA), and the biologics industry stakeholders are aware of the need to update the list of viruses of concern along with virus testing requirements for animal ingredients used in manufacturing biologics.

Gamma irradiation at doses >30 kilogray (kGy) is considered effective for most large and medium-size viruses[32], but may not be effective for certain medium or small porcine viruses such as polyomaviruses, parvoviruses, and circoviruses. Other inactivation methods including heat, ultraviolet light, and low or high pH exposure are also used as barrier treatments to inactivate viruses.[34] In particular, it appears that UV light treatment is effective in inactivating parvoviruses and circoviruses, but not polyomaviruses.[35]

It should be noted that neither the US Pharmacopeia (USP)[36] nor the European Pharmacopoeia (Ph. Eur.)[37] have developed specific monographs for porcine serum, while they have for bovine serum (USP 1024 and Ph. Eur. 2262), fetal bovine serum (USP 90), and trypsin (USP 29 and Ph. Eur. 0694).

6. Conclusions

The presence of ASF in Europe, China, and a growing number of its neighboring countries (currently 12) puts the rest of the world at high risk for importing the disease.

The geographic distribution of viruses of importation concern, like ASFV, can change very quickly, putting at risk conventional sources of porcine-derived products.

Safe sources of porcine serum, porcine trypsin, and other porcine products needed for the manufacture of biologics, research, and other biomedical applications, can be obtained from USDA and EU-approved origins.

References

[1] World Organization for Animal Health (OIE). The World Animal Health Information System (WAHIS). *African swine fever disease distribution map*. https://www.oie.int/wahis_2/public/wahid.php/Diseaseinformation/Diseasedistributionmap?disease_type_hidden=&disease_id_hidden=&selected_disease_name_hidden=&disease_type=0&disease_id_terrestrial=12&species_t=0&disease_id_aquatic=-999&species_a=0&sta_method=semesterly&selected_start_year=2020&selected_report_period=1&selected_start_month=1&date_submit=OK

[2] US Government Publishing Office. Code of Federal Regulations (CFR). 9 CFR 94. *Title 9, Animals and Animal Products, Chapter 1, Animal and Plant Health Inspection Service, Department of Agriculture, Subchapter D, Exportation and Importation of Animals (Including Poultry) and Animal Products. Part 94 – Foot-and-Mouth Disease, Newcastle Disease, Highly Pathogenic Avian Influenza, African Swine Fever, Classical Swine Fever, Swine Vesicular Disease, and Bovine Spongiform Encephalopathy: Prohibited and Restricted importations*. 15 Oct 2019. https://ecfr.io/Title-09/pt9.1.94

[3] European Union (EU). *Commission Regulation No 294/2013 of 14 March 2013 amending and correcting Regulation (EU) No 142/2011 implementing Regulation (EC) No 1069/2009 of the European Parliament and of the Council laying down health rules as regards animal by-products and derived products not intended for human consumption and implementing Council Directive 97/78/EC as regards certain samples and items exempt from veterinary checks at the border under that Directive with EEA relevance*. OJ L 98, 6.4.2013, p 1–57. https://eur-lex.europa.eu/eli/reg/2013/294/oj

[4] World Organization for Animal Health (OIE). The World Animal Health Information System (WAHIS). *Animal health situation*. https://www.oie.int/wahis_2/public/wahid.php/Countryinformation/Animalsituation

[5] World Organization for Animal Health (OIE). *Animal health in the world*. Official disease status. https://www.oie.int/animal-health-in-the-world/official-disease-status/

[6] World Organization for Animal Health (OIE). *Animal health in the world*. Self-declared disease status. https://www.oie.int/animal-health-in-the-world/self-declared-disease-status/

[7] United States Department of Agriculture (USDA), Food Safety and Inspection Service (FSIS). *Eligible Countries and Products*. https://www.fsis.usda.gov/wps/portal/fsis/topics/international-affairs/importing-products/eligible-countries-products-foreign-establishments/eligible-countries-and-products

[8] Personal communication with USDA, APHIS, National Center for Import Export (NCIE) staff. 9 August 2018.

[9] European Union (EU). *Commission Regulation (EU) No 206/2010 of 12 March 2010 laying down lists of third countries, territories or parts thereof authorized for the introduction into the European Union of certain animals and fresh meat and the veterinary certification requirements*. OJ L 73, 20.3.2010, p 1–121. https://eur-lex.europa.eu/eli/reg/2010/206/oj

[10] United States Department of Agriculture (USDA), Animal and Plant Health Inspection Service (APHIS). *Animal health status of regions*. https://www.aphis.usda.gov/aphis/ourfocus/animalhealth/animal-and-animal-product-import-information/animal-health-status-of-regions

[11] The Center for Food Security and Public Health, Iowa State University. *African swine fever*. June 2019. http://www.cfsph.iastate.edu/Factsheets/pdfs/african_swine_fever.pdf

[12] Center for Food Security and Public Health, Iowa State University. *Classical swine fever*. October 2015. http://www.cfsph.iastate.edu/Factsheets/pdfs/classical_swine_fever.pdf

[13] World Organization for Animal Health (OIE). The World Animal Health Information System (WAHIS). *List of countries by disease situation. African swine fever*. https://www.oie.int/wahis_2/public/wahid.php/Diseaseinformation/statuslist

[14] World Organization for Animal Health (OIE). *Animal health in the world. African swine fever*. https://www.oie.

int/en/animal-health-in-the-world/information-on-aquatic-and-terrestrial-animal-diseases/african-swine-fever/reports-on-asf/

[15] Dee SA *et al*. Survival of viral pathogens in animal feed ingredients under transboundary shipping models. *PLoS ONE*, 2018, 13(3), e0194509. https://doi.org/10.1371/journal.pone.0194509

[16] Queensland Government, Business Queensland. *Restricted invasive animals. Feral pig*. https://www.business.qld.gov.au/industries/farms-fishing-forestry/agriculture/land-management/health-pests-weeds-diseases/pests/invasive-animals/restricted/feral-pig

[17] Statista. *Number of pigs worldwide in 2018, by leading country (in million head)*. https://www.statista.com/statistics/263964/number-of-pigs-in-selected-countries/

[18] Nieto-Munoz S. *Feds seize 1 million lbs. of pork smuggled from China to N.J. port amid African swine fever outbreak*. 15 March 2019. https://www.nj.com/news/2019/03/feds-seize-1-million-lbs-of-pork-smuggled-from-china-to-nj-port-amid-african-swine-fever-outbreak.html

[19] World Organization for Animal Health (OIE). *Classical swine fever*. https://www.oie.int/fileadmin/Home/eng/Animal_Health_in_the_World/docs/pdf/Disease_cards/CLASSICAL_SWINE_FEVER.pdf

[20] World Organization for Animal Health (OIE). *Animal health in the world. Official disease status. Foot and mouth disease (FMD). OIE members' official FMD status map*. https://www.oie.int/en/animal-health-in-the-world/official-disease-status/fmd/en-fmd-carte/

[21] World Organization for Animal Health (OIE). *Vesicular stomatitis*. https://www.oie.int/fileadmin/Home/eng/Animal_Health_in_the_World/docs/pdf/Disease_cards/VESICULAR_STOMATITIS.pdf

[22] US Department of Agriculture (USDA), Animal and Plant Health Inspection Service (APHIS), Veterinary Services (VS). *2019 vesicular stomatitis virus (VSV) situation report*. 6 January 2020. https://www.aphis.usda.gov/animal_health/downloads/animal_diseases/vsv/sitrep-01-06-20.pdf

[23] World Organization for Animal Health (OIE). *Swine vesicular disease*. https://www.oie.int/fileadmin/Home/eng/Animal_Health_in_the_World/docs/pdf/Disease_cards/SWINE_VESICULAR_DISEASE.pdf

[24] US Government Publishing Office. Code of Federal Regulations (CFR). 9 CFR 121.3 and 121.4. *VS select agents and toxins and Overlap select agents and toxins*. https://www.ecfr.gov/cgi-bin/retrieveECFR?gp=1&SID=b9126e9fba23e3e-7933354a1d2630d72&ty=HTML&h=L&n=9y1.0.1.5.58&r=PART#se9.1.121_13

[25] Food and Agriculture Organization of the United Nations (FAO). Thirty-seventh session of the FAO conference. *Declaration of global freedom from rinderpest*. Rome, Italy. 25 June–2 July 2011. http://www.fao.org/3/i3366e/i3366e.pdf

[26] World Organization for Animal Health (OIE). *Rinderpest portal. 2011 – Global freedom from rinderpest*. https://www.oie.int/for-the-media/rinderpest/

[27] Food and Agriculture Organization of the United Nations (FAO). *Peste des petits ruminants*. http://www.fao.org/ppr/en/

[28] World Organization for Animal Health (OIE). *Rift valley fever*. https://www.oie.int/fileadmin/Home/eng/Animal_Health_in_the_World/docs/pdf/Disease_cards/RIFT_VALLEY_FEVER.pdf

[29] US Government Publishing Office. Code of Federal Regulations (CFR). 9 CFR 113. *Chapter 1, subchapter E – viruses, serums, toxins, and analogous products; organisms and vectors – standard requirements*. See 9 CFR 113.46, 113.47, 113.53(c) and 113.53(d). https://ecfr.io/Title-09/cfr113_main

[30] United States Department of Agriculture (USDA), Animal and Plant Health Inspection Service (APHIS), Veterinary Services, Center for Veterinary Biologics (CVB). *Notice No 18-05. Detection of Senecavirus A in veterinary biological products*. 17 May 2018. https://www.aphis.usda.gov/animal_health/vet_biologics/publications/notice_18_05.pdf

[31] European Medicines Agency (EMA), Committee for Medicinal Products for Human Use (CHMP). EMA/CHMP/BWP/814397/2011. *Guideline on the use of porcine trypsin used in the manufacture of human biological medicinal products*. 20 February 2014. https://www.ema.europa.eu/en/documents/scientific-guideline/guideline-use-porcine-trypsin-used-manufacture-human-biological-medicinal-products_en.pdf

[32] Plavsic M, Nims R, Wintgens M, Versteegen R. Gamma irradiation of animal serum: validation of efficacy for pathogen reduction and assessment of impacts on serum performance. *BioProcess J*, 2016; 15(2): 12–21. https://doi.org/10.12665/J152.Plavsic

[33] Marcus-Sekura C, Richardson JC, Harston RK, Sane N, Sheets RL. Evaluation of the human host range of bovine and porcine viruses that may contaminate bovine serum and porcine trypsin used in the manufacture of biological products. *Biologicals*, 2011; 39(6): 359–69. https://doi.org/10.1016/j.biologicals.2011.08.003

[34] Nims R, and Plavsic M. Inactivation of calicivi-ruses. *Pharmaceuticals*, 2013; 6(3): 358–92. https://doi.org/10.3390%2Fph6030358

[35] Nims R, Plavsic M. Identification of worst-case model viruses for selected viral clearance steps. *BioProcess J*, 2014; 13(2): 6–13. https://doi.org/10.12665/J132.Nims

[36] The United States Pharmacopeial Convention (USP). *Biologics raw and ancillary materials standards*. https://www.usp.org/biologics/raw-ancillary-materials

[37] European Directorate for the Quality of Medicines (EDQM). *European Pharmacopoeia (Ph. Eur.) 10th edition*. https://www.edqm.eu/en/european_pharmacopoeia_10th_edition

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