Persistence of african swine fever virus in environment and the effect of chemical disinfectants

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OBJECTIVES

1. important characteristics of ASF virus particles
2. persistence of ASFV in environment
3. efficacy and use of disinfectants against ASFV in agriculture

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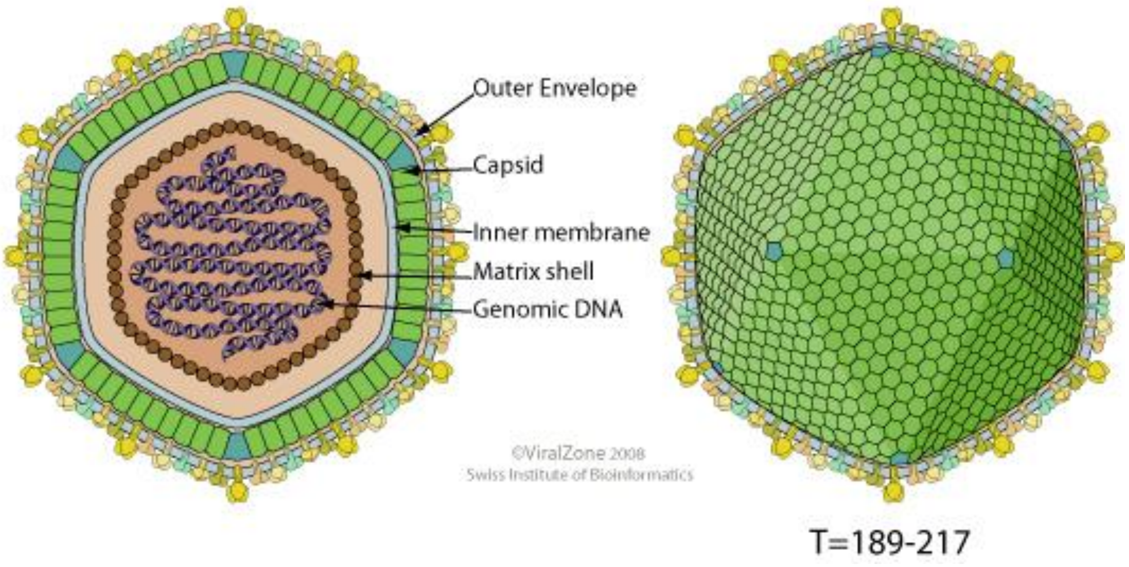
Diagram of extracellular virion



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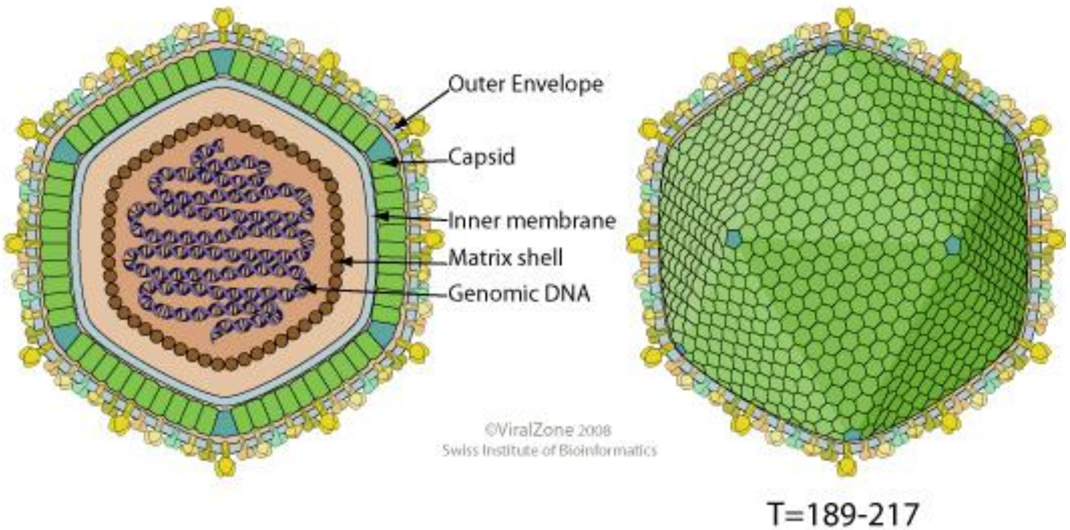
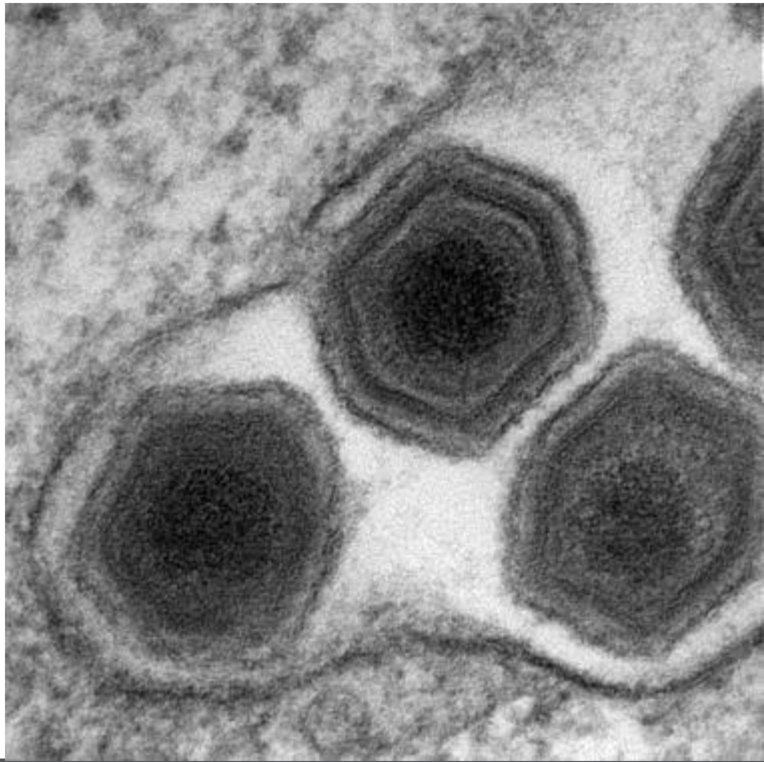


175-215 nm in diameter

Extracellular virion

Alonso et al. *Journal of General Virology*



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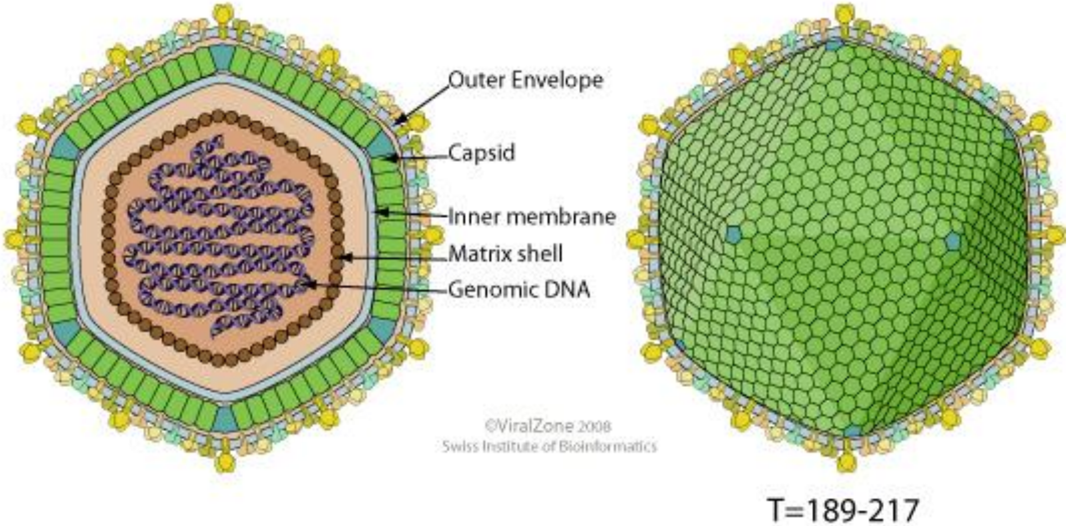

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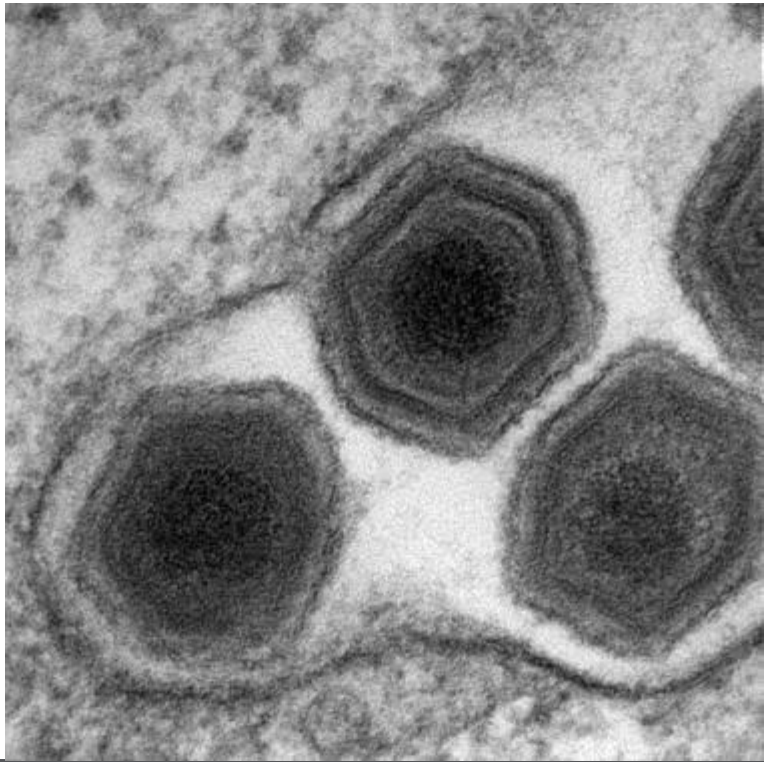
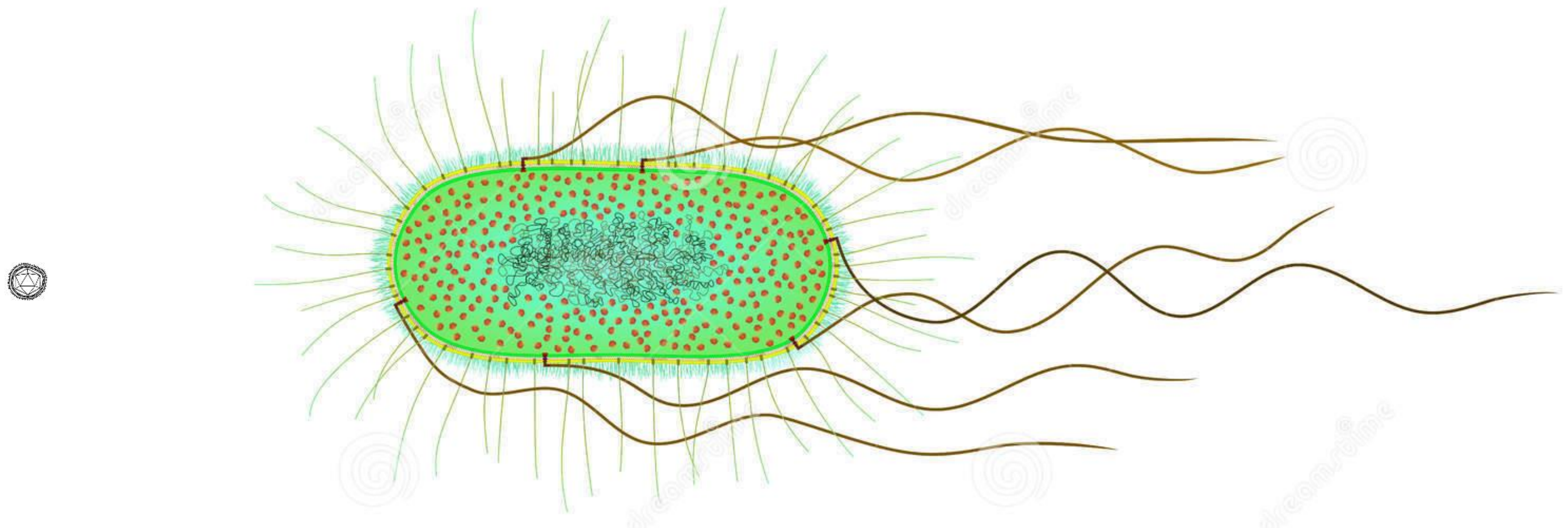


Genome

- single molecule of ds DNA
- 170 – 194 kbp

Viruses

1. are smaller than other microorganisms
2. have a more simple structure
3. lack metabolic activity



Viruses

1. are smaller than other microorganisms
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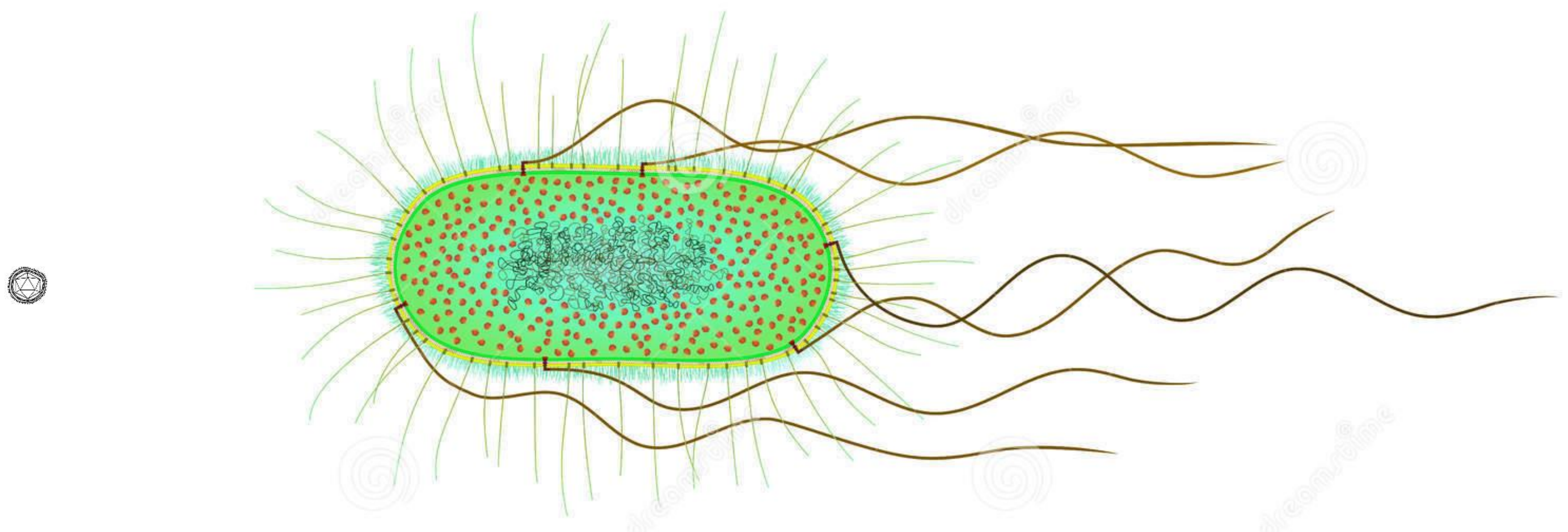
3. lack metabolic activity

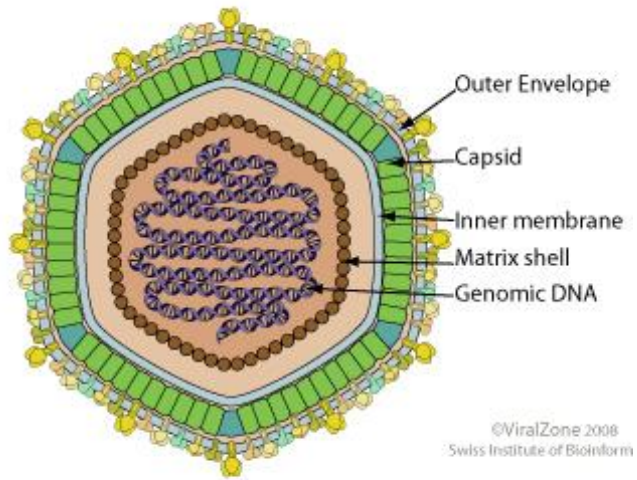
tricky target for biocides



Target sites for chemical disinfectants on virus particle:

1. envelope (if presented)

- large amount of lipids
- derived from host cell or nuclear membrane



2. capsid

- consists of proteins
- responsible for virus shape and genome protection
- structural damage of capsid might not always represent a loss of viral infectivity

3. viral genome

- viral inactivation is complete only if NA is destroyed

Sensitivity to chemical disinfectants:

Klein-DeForest category	Solubility	Chemical structure	Sensitivity
Lipid	Lipophilic (envelope)	Nucleic acid + capsid + envelope	**Obvious**
			Ortho- and Paramyxo
			RSV
			Herpes
			HIV
Nonlipid	Hydrophilic (no envelope)	Nucleic acid + capsid	**Slight**
			Polio
			Coxsackie
			Rhino
Nonlipid	Intermediate (capsomeric lipophilicity)	Nucleic acid + capsid	**Moderate**
			Adeno
			Reo
			Rota

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Prince, H.N. and Prince, D.L. Principles of viral control and transmission. *In:* S.S.Block (ed.) Disinfection, sterilization, and Preservation, Fifth Edition. Lippincot Williams and Wilkins 2001.

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Klein-DeForest category	Solubility	Chemical structure	Sensitivity
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Nonlipid	Hydrophilic (no envelope)	Nucleic acid + capsid	**Slight** Polio Coxsackie Rhino
Nonlipid	Intermediate (capsomeric lipophilicity)	Nucleic acid + capsid	**Moderate** Adeno Reo Rota

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Klein-DeForest category	Solubility	Chemical structure	Sensitivity
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			RSV
			Herpes

ASFV

In the presence of high organic load, proteins or mucin have protective effect on virus particles. Viruses may be more difficult to inactivate.

			Coxsackie
			Rhino
Nonlipid	Intermediate (capsomeric lipophilicity)	Nucleic acid + capsid	**Moderate**
			Adeno
			Reo
			Rota

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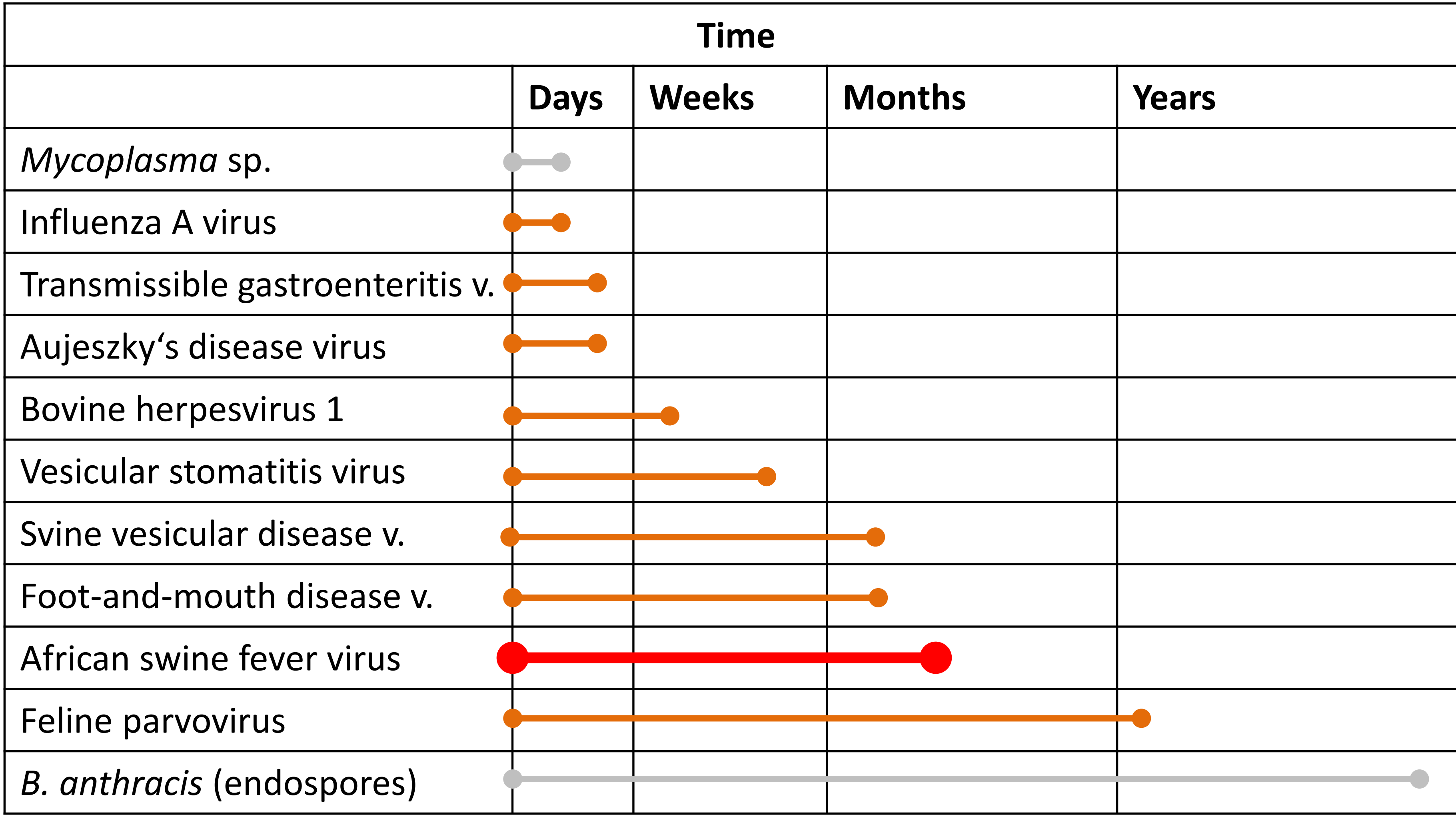
Persistence of viruses in environment:

- survival of viruses is **influenced by many factors** (initial number of virus particles shed, nature and amount of organic matter present, temperature, pH, humidity, UV light...)

Persistence of viruses in environment:

- survival of viruses is **influenced by many factors** (initial number of virus particles shed, nature and amount of organic matter present, temperature, pH, humidity, UV light...)
- role of the environment in virus transmission is **difficult to assess**

Estimated survival time of animal pathogens in environment at ambient temperature:



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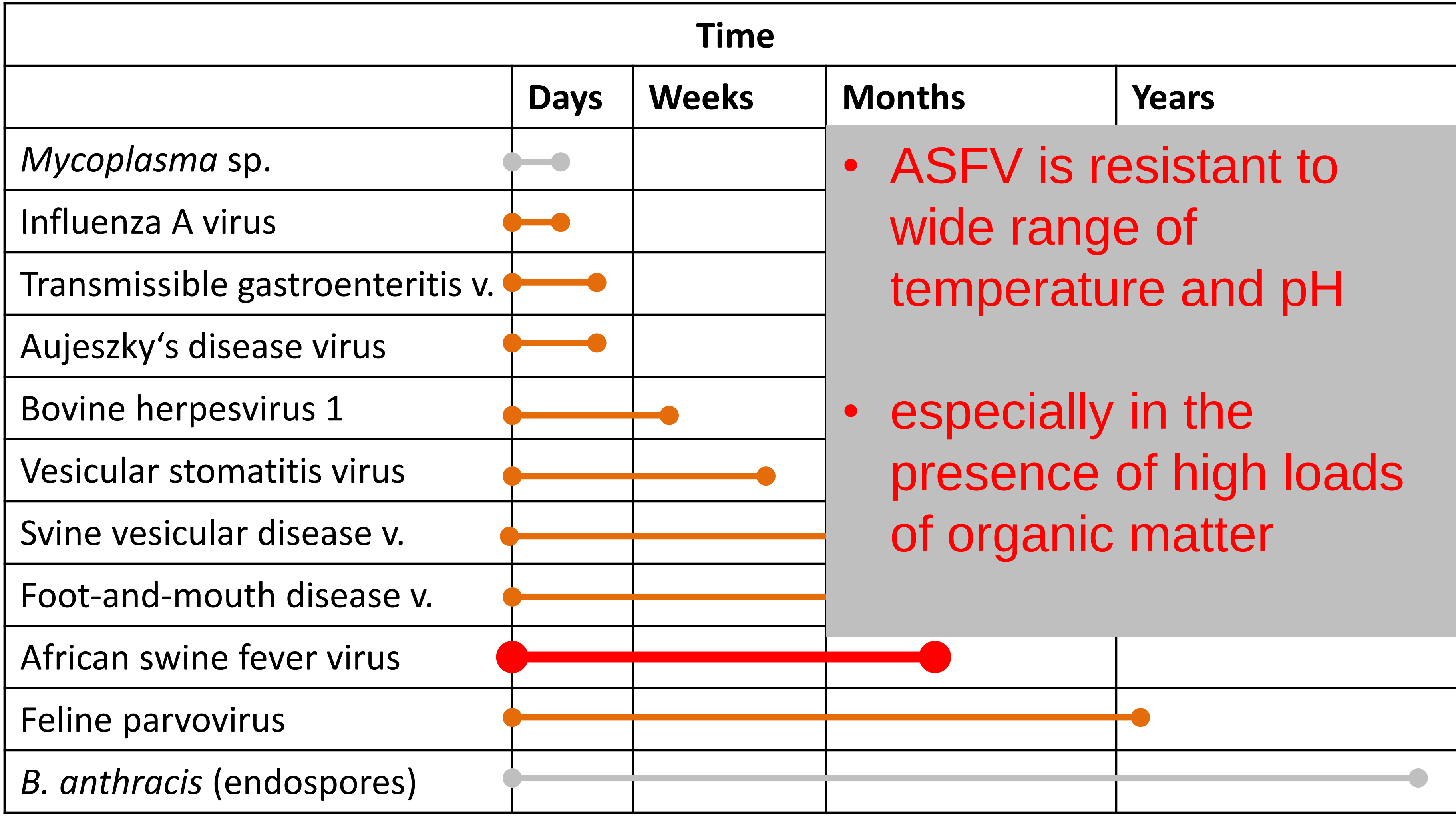

- ASFV is resistant to wide range of temperature and pH
- especially in the presence of high loads of organic matter

Survival of viruses:

AIR

- depend on virus, relative humidity, and temperature

Surviving of viruses:

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- depend on virus, relative humidity, and temperature
- viruses with little or no lipid content are usualy stable at high RH
- viruses with higher lipid content are usually stable at low RH

Survival of viruses:

AIR

- depend on virus, relative humidity, and temperature
- viruses with little or no lipid content are usualy stable at high RH
- viruses with higher lipid content are usually stable at low RH
- aerosol transmission of currently circulating ASFV was demonstrated

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ELSEVIER

Transmission of African swine fever virus from infected pigs by direct contact and aerosol routes

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Survival of viruses:

WATER

- in general, water comprise possible source of viral infection of farm animals

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- ASFV survived in a lake water for up to 176 days during winter

Survival of viruses:

WATER

- in general, water comprise possible source of viral infection of farm animals

- ASFV strain Georgia 2007 can be **easily transmitted** orally through consumption of liquid
- the median infectius dose was **10^1 $TCID_{50}$**

Infectious Dose of African Swine Fever Virus When Consumed Naturally in Liquid or Feed

Megan C. Niederwerder, Ana M.M. Stoian, Raymond R.R. Rowland, Steve S. Dritz, Vlad Petrovan, Laura A. Constance, Jordan T. Gebhardt, Matthew Olcha, Cassandra K. Jones, Jason C. Woodworth, Ying Fang, Jia Liang, Trevor J. Hefley

African swine fever virus (ASFV) is a contagious, rapidly spreading, transboundary animal disease and a major threat to pork production globally. Although plant-based feed has been identified as a potential route for virus introduction onto swine farms, little is known about the risks for ASFV transmission in feed. We aimed to determine the minimum and median infectious doses of the Georgia 2007 strain of ASFV through oral exposure during natural drinking and feeding behaviors. The minimum infectious dose of ASFV in liquid was 10^0 50% tissue culture infectious dose ($TCID_{50}$), compared with 10^4 $TCID_{50}$ in feed. The median infectious dose was $10^{1.0}$ $TCID_{50}$ for liquid and $10^{6.8}$ $TCID_{50}$ for feed. Our findings demonstrate that ASFV Georgia 2007 can easily be transmitted orally, although higher doses are required for infection in plant-based feed. These data provide important information that can be incorporated into risk models for ASFV transmission.

African swine fever virus (ASFV) is an emerging threat to swine production in North America and Europe. During the past decade, ASFV has spread into Eastern Europe and Russia (*1*,*2*) and most recently into China (*3*,*4*) and Belgium (*5*). Disease caused by ASFV is characterized by severe disseminated hemorrhage, and case-fatality rates approach 100% (*6*). The virus is a member of the *Asfarviridae* family and is the only known vectorborne DNA virus (*7*). Challenges to disease control include the lack of available vaccines and the potential for ASFV to become endemic in feral swine and ticks (*8*). Because no effective vaccine or treatment exists, preventing ASFV introduction is the primary goal of disease-free countries. Mitigation strategies during an African swine fever (ASF) outbreak are centered around restricting pig movement and conducting large-scale culling of infected herds. It is estimated that the introduction of ASFV into the United States would cost producers >$4 billion in losses (*9*).

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Historical outbreaks, including the introduction of ASFV into the Caucasus region in 2007 and subsequent spread into Russia, have been attributed to feeding contaminated pork products (*1*) or direct contact with pigs (*10*). ASFV survives in meat and blood at room temperature for several months (*11*,*12*) and is resistant to temperature and pH extremes (*13*). Molecular characterization of the more recent ASFV incursions into China (*4*) and Siberia (*14*) demonstrate similarity in viral isolates to the Georgia 2007 strain of ASFV. These outbreaks have occurred in herds separated by thousands of kilometers (*15*). For example, ASFV spread ≈2,100 km from the city Shenyang in northern China to the city Wenzhou, south of Shanghai, in ≈3 weeks (*16*). Also, an ASFV incursion has been reported recently in a large-scale, high-biosecurity farm in Romania (*17*). Contaminated water from the Danube River has been implicated in introducing ASF onto the ≈140,000-pig breeding farm (*18*). Contaminated feed as a transmission vehicle for introducing transboundary animal diseases onto high-biosecurity swine operations has been recognized as a major risk factor since the introduction of porcine epidemic diarrhea virus into the United States in 2013 (*19–24*). The lesson learned from porcine epidemic diarrhea virus underscores the need to quantitate the risk that feed plays in the introduction of other transboundary animal diseases. Nonetheless, data defining the risk for ASFV transmission through feed or feed ingredients are limited.

In 2014, the introduction and spread of ASFV in Latvia was associated with the feeding of virus-contaminated fresh grass or crops to naive pigs (*25*). Furthermore, recent work has demonstrated that ASFV survives in feed ingredients, such as conventional soybean meal, organic soybean meal, soy oil cake, and choline, under conditions simulating trans-Atlantic shipment from Eastern Europe to the United States (*21*). These reports suggest that the spread of ASFV might be attributed to less-recognized transmission routes, such as feed or water.

Survival of viruses:

FEED

- feeding of contaminated meat product or food waste is significant source of infection

Survival of viruses:

FEED

- feeding of contaminated meat product or kitchen waste is important source of ASFV

Survival of ASFV in different types of meat products:

raw meat (+4°C)	several months
offal	several months
skin fat	300 days
salted dry meat	120 days
ham in brine	180 days
frozen meat/carcasses	years

Survival of viruses:

FEED

- infective ASFV was recovered from 9 feed ingredients after simulated manufacturing and shipping process

Ingredient	SVA (FMDV)	ASFV	PSV (SVDV)	PEDV	FCV (VESV)	PCV2
Soybean meal-Conventional	(+)	(+)	(+)	(+)	(+)	(-)
Soybean meal-Organic	(-)	(+)	(+)	(+)	(-)	(-)
Soy oil cake	(+)	(+)	(+)	NT	(-)	(-)
DDGS	(+)	(-)	(-)	NT	(-)	(-)
Lysine	(+)	(-)	(+)	(+)	(+)	(+)
Choline	(+)	(+)	(-)	(+)	(-)	(+)
Vitamin D	(+)	(-)	(+)	(+)	(-)	(+)
Moist cat food	(+)	(+)	(+)	NT	(-)	(-)
Moist dog food	(+)	(+)	(+)	NT	(-)	(-)
Dry dogfood	(+)	(+)	(+)	NT	(-)	(-)
Pork sausage casings	(+)	(+)	(+)	NT	(+)	(-)
Complete feed (+ control)	(+)	(+)	(+)	NT	(+)	(+)
Complete feed (- control)	(-)	(-)	(-)	(-)	(-)	(-)
Stock virus control	(-)	(+)	(-)	(-)	(-)	(-)

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RESEARCH ARTICLE

Survival of viral pathogens in animal feed ingredients under transboundary shipping models

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Abstract

The goal of this study was to evaluate survival of important viral pathogens of livestock in animal feed ingredients imported daily into the United States under simulated transboundary conditions. Eleven viruses were selected based on global significance and impact to the livestock industry, including Foot and Mouth Disease Virus (FMDV), Classical Swine Fever Virus (CSFV), African Swine Fever Virus (ASFV), Influenza A Virus of Swine (IAV-S), Pseudorabies virus (PRV), Nipah Virus (NiV), Porcine Reproductive and Respiratory Syndrome Virus (PRRSV), Swine Vesicular Disease Virus (SVDV), Vesicular Stomatitis Virus (VSV), Porcine Circovirus Type 2 (PCV2) and Vesicular Exanthema of Swine Virus (VESV). Surrogate viruses with similar genetic and physical properties were used for 6 viruses. Surrogates belonged to the same virus families as target pathogens, and included Senecavirus A (SVA) for FMDV, Bovine Viral Diarrhea Virus (BVDV) for CSFV, Bovine Herpesvirus Type 1 (BHV-1) for PRV, Canine Distemper Virus (CDV) for NiV, Porcine Sapelovirus (PSV) for SVDV and Feline Calicivirus (FCV) for VESV. For the remaining target viruses, actual pathogens were used. Virus survival was evaluated using Trans-Pacific or Trans-Atlantic transboundary models involving representative feed ingredients, transport times and environmental conditions, with samples tested by PCR, VI and/or swine bioassay. SVA (representing FMDV), FCV (representing VESV), BHV-1 (representing PRV), PRRSV, PSV (representing SVDV), ASFV and PCV2 maintained infectivity during transport, while BVDV (representing CSFV), VSV, CDV (representing NiV) and IAV-S did not. Notably, more viruses survived in

Survival of viruses:

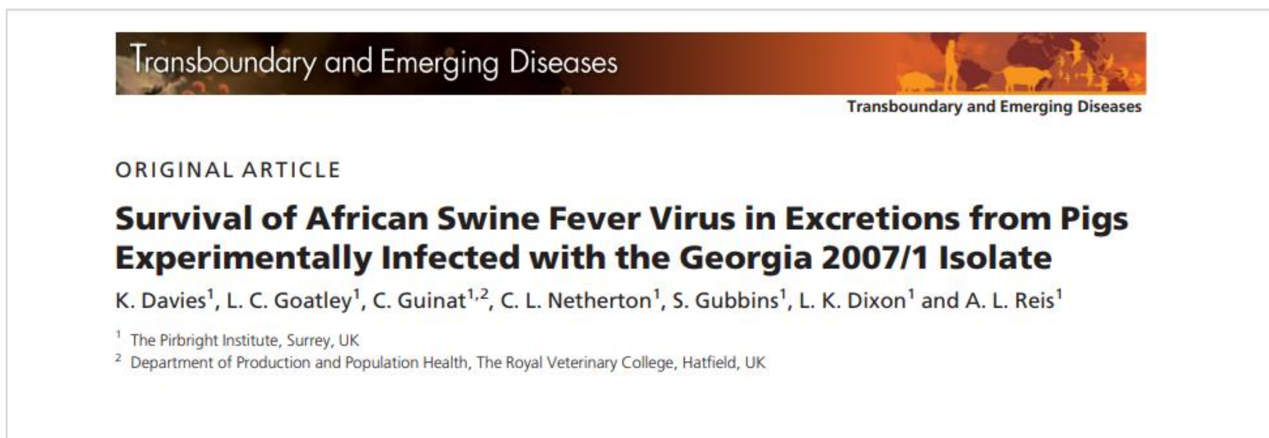
BLOOD

- ASFV is shed in blood in high concentration
- persists in blood for 15 weeks at RT, over a year at +4°C, and years when frozen
- contaminated soil, surfaces, tools, equipment could represent important source of infection

Survival of viruses:

EXCRETIONS

- ASFV is shed in both urine and faeces
- survival is affected by temperature
- genotype II ASFV is more stable in urine than in faeces
- urine would be estimated to remain infectious for 15 days at 4°C



Transboundary and Emerging Diseases

Transboundary and Emerging Diseases

ORIGINAL ARTICLE

Survival of African Swine Fever Virus in Excretions from Pigs Experimentally Infected with the Georgia 2007/1 Isolate

K. Davies[1], L. C. Goatley[1], C. Guinat[1,2], C. L. Netherton[1], S. Gubbins[1], L. K. Dixon[1] and A. L. Reis[1]

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Survival of viruses:

SOIL

- environmental influences on soil survival involve temperature, light, moisture content, pH, salt species, soil adherence, organic matter, and hydraulic conditions
- ASFV – more research is needed to understand survival in soil

Survival of viruses:

SURFACES

- viruses in general show greater survival on surfaces
- virus particles often exists in clumps protected with organic matter

Survival of viruses:

SURFACES

- viruses in general show greater survival on surfaces
- virus particles often exists in clumps protected with organic matter
- any **contaminated surface** represents **source of infection**
- **the appropriate use of chemical disinfectants interrupts disease transmission from surface**

Proper use of chemical disinfectants:

Disinfection of surface OR animal facility			
Cleaning	removal of bedding, faeces, feed, dust immediately after removal of animals		surfaces are visualy **clean**
	dry cleaning	brushing, scraping...	
	wet cleaning	soaking with water	
		washing with water and detergents	
		final rinse with water	
Disinfection	application of disinfectant		surfaces are **disinfected** = most of recognised pathogens is destroyed and surface is not longer a source of infection

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Juzskiewicz, M. et al. Characteristics of selected active substances used in disinfectants and their virucidal activity against ASFV. J Vet Res 2019, 63:17-25.

Characteristic of optimal disinfectants for use in veterinary medicine:

- wide antimicrobial spectrum
- absence of irritancy, toxicity, teratogenicity, mutagenicity, and carginogenicity
- compatibility with a wide range of chemicals
- absence of corrosiveness
- retention of activity in the presence of organic matters
- stability at ambient temperature with a long shelf life
- effective over wide range of temperature
- inexpensive and easily available
- nonpolluting for groung water or air
- biodegradable

Quinn, P.J. and Markey, B.K. Disinfection and disease prevention in veterinary medicine. *In:* S.S.Block (ed.) Disinfection, sterilization, and Preservation, Fifth Edition. Lippincot Williams and Wilkins 2001.

The choice of disinfectants:

Susceptibility to chemical disinfectants	Microorganisms	Effective disinfectants
Highly susceptible	Mycoplasmas	
Susceptible	Gram-positive bacteria	Alcohols, aldehydes, alkalis, biguanides, ethylene oxide, halogens, ozone, peroxygen compounds, some phenols, some quarternary ammonium compounds
	Enveloped viruses	
	Gram-negative bacteria	
	Fungal spores	
Resistant	Non-enveloped viruses	
	Mycobacteria	
Highly resistant	Bacterial endospores	
	Protozoal oocysts	
Extremely resistant	Prions	

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Mechanisms of action of virucidal agents:

Category	**Agent**
Denaturants = physically disrupt protein or lipid structures	Quarternary ammonium compounds*
	Chlorhexidine
	Phenolics
	Acids
	Basis
	Alcohols
Reactants = form or break covalent bonds	**Aldehydes**
	Enzymes
Oxidants = increase positive valence of carbon, sulphur, and nitrogen	**Halogens**
	Hydrogen peroxide
	Ozone

*virucid and detergent

Aldehydes:

- wide antimicrobial activity
- act relatively slowly
- toxic, irritant and potentially carcinogenic

Glutaraldehyde

- affected minimally by the presence of organic matter
- pH and temperature are of critical importance
- noncorrosive, does not damage plastics and rubber

Formaldehyde

- gas (fumigation) or aqueous solution (formalin)
- in the presence of organic matter is less effective than glutaraldehyde

Chlorine compounds:

- potent virucides for surfaces, equipment, buildings, vehicles
- low toxicity at effective concentrations
- easy to use
- relatively low cost
- in concentrated forms usually unstable (affected by light and heat)

Sodium hypochlorite

- **one of the most widely used disinfectants**
- fast-acting, non-staining, and inexpensive
- corrosive
- **easily inactivated by organic matter**
- optimal activity at pH 5

Chlorine dioxide, Chloramine-T ...

Iodine compounds:

- less chemically reactive than chlorine compounds
- more active in the presence of organic matter
- **iodophors** = iodine is complexed with surface active compounds or polymers (polyvinylpyrrolidone = povidone-iodine)

Iodophors

- broad range of antimicrobial aktivity
- effective in the presence of organic matter and at both low and high temperatures
- less active in alkaline pH

Factors that may contribute to the failure of disinfection:

Disinfectant factors
• choice of innefective disinfectant • disinfectants too dilute • insufficient contact time allowed • temperature too low for optimal activity
Environmental factors
• residual organic matter due to inadequate cleaning • lack of contact with disinfectants because of unsuitable surface • improper application to surfaces or equipment
Other factors
• reintroduction of infectious agent

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