

Persistence of Disease Agents in Carcasses and Animal Products

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**Report for Animal Health
Australia**

by

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Updated by

Herd Health Pty Ltd

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Table of Contents

Persistence of Disease Agents in Carcasses and Animal Products.....	1
Introduction.....	4
Purpose of Consultancy	4
Methodology	6
Notes on the Report	8
Review of Disease Agents	9
Bunyaviridae	9
Nairobi sheep disease.....	9
Rift Valley fever	11
Coronaviridae.....	13
Porcine Epidemic Diarrhoea Virus	13
Flaviviridae	16
Tickborne encephalitides	18
Wesselsbron disease.....	20
Orthomyxoviridae	21
Avian influenza	21
Equine influenza	26
Swine influenza.....	28
Paramyxoviridae	30
Hendra virus.....	30
Menangle virus (porcine paramyxovirus).....	32
Newcastle disease	33
Nipah virus.....	37
Peste des petits ruminants	39
Rinderpest	42
Picornaviridae	45
Foot-and-mouth disease	45
Swine vesicular disease.....	52
Teschen disease (porcine polioencephalomyelitis).....	55
Poxviridae	57
Lumpy skin disease.....	57
Sheep pox / Goat pox	60
Reoviridae	64
African horse sickness	64

Bluetongue	66
Equine encephalosis	69
Retroviridae	70
Jembrana disease	70
Maedi-visna	72
Pulmonary adenomatosis	75
Rhabdoviridae	77
Australian lyssavirus	77
Rabies	78
Vesicular stomatitis	81
Togaviridae	84
Getah virus disease	84
Porcine reproductive and respiratory syndrome	85
Western, Eastern and Venezuelan equine encephalomyelitis	89
Other viruses	91
African swine fever	91
Aujeszky's disease	95
Borna disease	99
Classical swine fever	101
Infectious bursal disease (hypervirulent form)	106
Transmissible gastroenteritis	110
Vesicular exanthema	114
Prions	117
Bovine spongiform encephalopathy and scrapie	117
Bacteria, mycoplasmas and fungi	123
Anthrax	123
Heartwater	127
Brucellosis (<i>B. abortus</i>)	128
Brucellosis (<i>B. melitensis</i>)	132
Bovine tuberculosis	136
Haemorrhagic septicaemia	141
Glanders	143
Contagious equine metritis	145
Potomac fever	147
Contagious bovine pleuropneumonia	148
Epizootic lymphangitis	150
Protozoa	151
Surra	151
Dourine	153
East Coast fever	155
Equine babesiosis (piroplasmosis)	156
Multicellular parasites	157
Sheep scab	157
Screw-worm fly	159
Trichinellosis	161
Appendix 1	165
Example search strategy – 2001 report	165
Example search strategy – 2017 review	166

Introduction

Purpose of Consultancy

The purpose of this consultancy was to provide information to enable Commonwealth and State/Territory veterinary, environmental and public health officials to review the disposal methods available for animals and animal products in Australia and to revise the animal disposal manual of AUSVETPLAN.

Terms of reference for the original consultancy were as follows:

For each of the 63 disease agents identified in the Cost Sharing Agreement, their persistence in carcasses and animal products, both after slaughter and when the carcasses and animal products are disposed of or treated by the methods identified in the scoping workshop of 2-3 July 2001, is to be determined.*

A listing of all 63 disease agents is to be constructed in a user-friendly, but scientifically accurate, format detailing the minimum requirements for inactivation / destruction of the disease agent, designed to be used for the purpose of determining the most appropriate method of disposal of infected carcasses and animal products.

** These methods are:*

1. *Left in situ, i.e. no further treatment*
2. *Burial*
3. *Above-ground 'burial'*
4. *Aerobic decomposition*
5. *Burial at sea*
6. *Chilling*
7. *Rendering*
8. *Combustion.*

Terms of reference for the 2017 consultancy to update the report were as follows:

AHA seeks a report on the persistence of emergency animal disease agents in carcasses and animal products. This will inform risk assessment of possible disease transmission pathways and inform policy recommendations on appropriate disposal and/or treatment (including disinfection) methods for these and related items in an emergency animal disease outbreak.

For each disease listed under the EADRA, the report should include an overview of the agents's persistence at slaughter and through the following:

- *disposal processes: burial, burning, rendering, composting, anaerobic digestion, other*
- *methods (alkaline hydrolysis, leaving in situ, ocean disposal etc, treatment processes: exposure to changes in pH, temperature (chilling, freezing, 'cooking' and other heating methods), and any other relevant processes identified through research.*

This overview will be applied for the following products: carcasses (whole and dressed); meat and meat products; milk and milk products; eggs and egg products;

skin, hides and fibres; semen and embryos; and, faeces, urine and other bodily secretions.

For each disease listed under the EADRA (and corresponding aetiological agent), the report should also detail its susceptibility to an agreed range of disinfectants and the disinfectant's recommended application on an agreed range of different substrates relevant to a disease response. The final list of disinfectants and substrates to be included is subject to negotiation between AHA and the consultant. This work should draw on open sources of information - including existing AUSVETPLAN manuals, the OIE Terrestrial Animal Health Code and Manual of Diagnostic Tests and Vaccines for Terrestrial Animals, the technical fact sheets produced by the Center for Food Security and Public Health (CFSPH, Iowa State University), USDA-APHIS and the FAO. It should also draw on specific relevant scientific and technical papers published in the last 10 years.

The report should include:

- a short description of the methodology used and assumptions made*
- details of relevant experts contacted (eg in an appendix)*
- an overview of bio-control effectiveness by group of aetiological agent (grouping to be negotiated between AHA and the consultant)*
- for disposal and treatment processes, an agent-product-control matrix that logically maps bio-control effectiveness (details to be negotiated between AHA and the consultant)*
- an overview of disinfectant susceptibility by group of aetiological agent (grouping to be negotiated between AHA and the consultant)*
- disinfectant susceptibility information for each agent in a disinfectant-substrate matrix to describe use patterns and specific precautions/contraindications (details to be negotiated between AHA and the consultant).*

The report is to be suitable for use as a consultation draft with the AUSVETPLAN Technical Review Group.

The consultant is also to provide an electronic database of references and relevant papers and provide electronic copies of literature cited. These should be presented in a form suitable for archiving, updating, disseminating and searching.

Methodology

2001 consultancy

The original consultancy was undertaken in four overlapping stages:

1. Review of microbiological and other texts for information relating to persistence and disposal methods for the 63 organisms.

Microbiological texts were generally found to be unrewarding. By far the most useful text for most of the agents studied in this review was *Infectious diseases of livestock with special reference to Southern Africa, vols I and II*, eds. Coetzer, J.A.W., Thomson, G.R., & Tustin, R.C., Oxford University Press, Cape Town, 1994.

Other texts commonly referred to were Geering, W.A., Forman, A.J., & Nunn, M.J. 1995, *Exotic diseases of animals: a field guide for Australian veterinarians*, Australian Government Publishing Service, Canberra, and Radostits, O.M., Gay, C.G., Blood, D.C., & Hinchcliff, K.W. (eds) 2000, *Veterinary medicine*, ninth edition, W. B. Saunders Company Ltd, London.

2. Detailed literature search.

A search was made of Medline (PubMed), CAB Abstracts, Agricola, Australian Bibliography of Agriculture (ABOA) and Current Contents databases for relevant papers (reviews, epidemiological articles) on each disease / agent. The searches were conducted by the Senior Librarian at the Victorian Institute of Animal Science. The search strategy was similar for each disease / agent, although more focused in the case of diseases such as foot-and-mouth disease. An example of the search strategy is given in Appendix 1.

Key reviews and recent references were acquired and checked for information not obtained in stage 1. It was outside the scope of the consultancy to obtain every reference identified as relevant. As far as possible throughout this report, original references are identified even where they have been cited from other papers. The source of the citation is also given.

During the review, extensive cross-checking was made against draft and final Import Risk Assessments (IRAs) prepared over the last two years by the Australian Quarantine and Inspection Service (AQIS) / Animal Biosecurity. The IRAs provide a very thorough review of the persistence and inactivation of a range of disease agents in selected products, and analysis of the risk of transmission by those products.

3. Telephone and/or e-mail contact with individual experts and other key persons.

Australian and international experts for most diseases were contacted directly. The experts were asked for suggestions on key references, to cross-check the literature reviews; and about information from unpublished or in-progress studies. Names were initially obtained from the Office International des Epizooties (OIE) web site. Others were identified from papers obtained through stages 1 and 2, or from referrals.

Contacting experts proved to be much more time-consuming than anticipated. Some experts were very difficult to contact, despite repeated telephone calls and e-mails, and a number were not reached within the timeframe of the consultancy.

4. Compilation of report.

This report was first delivered in December 2001.

2017 Revision

The 2017 update was conducted as follows:

Search results and referenced papers from the 2001/03 report were collated.

These papers were scanned and archived

A search of Web of Science for relevant publications published since 2000.

The search system was applied for each disease. Search results were dumped to file for incorporation into a reference database

Relevant papers were obtained and reviewed

The original report disease sheets were updated as required. This was completed in February 2017

The EADRA disease list (March 2017) was used for the report. Only diseases on the EADRA list are reported in this update.

Notes on the Report

1. This is a technical report focusing on the persistence and inactivation of disease agents. Greater emphasis has been placed on thorough review of the relevant literature than on drawing conclusions about disposal methods. A number of factors will contribute to determining the optimum disposal method including; effectiveness in halting transmission, cost, attitude to risk, ease of implementation, public perception, local factors (such as topography, accessibility, and native and feral fauna). These factors were outside the scope of this review.
2. There are many gaps in the knowledge about persistence and inactivation of certain disease agents in carcasses and animal products. In particular, there are very few primary studies on the inactivation of agents in disposal methods other than leaving in situ, incineration and burial. A number of the experts contacted commented on the paucity of information available.
3. Reasons for this lack of information include low relevance to the epidemiology or control for a number of the diseases examined. For example, for most of the vector-borne diseases, the vectors have appropriately received most of the attention. Information on persistence may also be a low priority to countries in which the disease is endemic and where efforts are therefore directed at control (e.g. vaccines) rather than stamping out. At the same time, there is reluctance to work with the agent in countries where it is exotic.
4. For each of the disease agents in the report, the following information is presented:
 - a. General characteristics of the agent and of the disease that are relevant to transmission via carcasses and animal products – including *in vitro* data on physical and chemical characteristics, survival in the environment, effective disinfectants, where the agent is found in the body, and how it is transmitted
 - b. Summaries of specific studies on the persistence and inactivation of the agent in products from the host, with some analysis and interpretation, including information from experts where applicable
 - c. References.

Review of Disease Agents

Bunyaviridae

Nairobi sheep disease

Agent:

Family Bunyaviridae, genus nairovirus

Agent type:

Virus

Persistence and inactivation:

General characteristics: Nairobi sheep disease virus is a relatively unstable agent. At its optimum pH range of 7.4-8.0 and with 2% serum, it has a half-life of 6.8 days at 0°C and 1.5 hours at 37°C. Stability is greater in citrated whole blood, where the presence of protein is protective: 66 days at 5°C, 33 days at 15-18°C, 42 hours at 37°C, 1.5 hours at 50°C, and 5 minutes at 60°C. NSDV is sensitive to lipid solvents and detergents and is rapidly inactivated outside the optimum pH range (Terpstra 1990, Montgomery 1917). The USDA (2003) consider Bunyaviruses to be susceptible to hypochlorite, phenolics and 2% glutaraldehyde. Other disinfectants may also be effective.

NSD is transmitted by ticks, mainly of the genus *Rhipicephalus* (Terpstra 1990).

Carcases and meat products: The pathogenesis of NSD includes a viraemic phase in which the virus spreads to most internal organs. No references were uncovered on persistence of NSDV in carcasses or muscle during the conduct of this review. The virus would be expected to disappear quickly from the carcass as the pH dropped with rigor mortis. AQIS (1999a) does not consider NSD to be a risk in imported meat products.

Davies (1988) noted that, in contrast to Rift Valley fever, there were no reports of humans becoming infected despite handling infected carcasses.

Milk and milk products: No reports were uncovered on the shedding of NSDV in milk. AQIS (1999b) does not regard milk products as posing a threat for the importation of NSD.

Skins, hides and fibres: No reports were uncovered on the contamination of skins, wool or hair by NSDV. In a draft assessment, AFFA (2001) has determined that skins and hides do not pose an import risk for NSD.

Semen/embryos: No reports were uncovered on the shedding of NSDV in semen or with embryos.

Faeces: Stock affected with NSD suffer an acute diarrhoea (Terpstra 1990), but there are no reports of virus being isolated from faeces.

References:

AFFA (Department of Agriculture Fisheries and Forestry – Australia) 2001, *Import risk analysis. Skins and hides. Draft report, August 2001*, AFFA, Canberra.

AQIS (Australian Quarantine and Inspection Service) 1999a, *Importation of sausage casings into Australia, import risk analysis, December 1999*, AQIS, Canberra.

AQIS (Australian Quarantine and Inspection Service) 1999b, *Import risk analysis: Importation of dairy products for human consumption*, AQIS, Canberra.

Davies, F.G. 1988, 'Nairobi sheep disease', in *The arboviruses: epidemiology and ecology. Vol III*, ed. Monath, T.P., CRC Press, Boca Raton, Florida, pp. 191-203.

Montgomery, R.E. 1917, 'On a tick-borne gastro-enteritis of sheep and goats occurring in British East Africa', *J Comp Pathol Ther*, vol. 30, pp. 28-58, cited in Terpstra 1990.

Terpstra, C. 1990, 'Nairobi sheep disease virus', in *Virus infections of ruminants*, eds. Z. Dinter and B. Morein, Elsevier Science Publishers B.V., Amsterdam, pp. 495-500.

Veterinary Services, Animal and Plant Health Inspection Service, USDA. 2003, 'Nairobi Sheep Disease Standard Operating Procedures: 1. Overview Of Aetiology And Ecology' in *Draft Foreign Animal Disease Preparedness and Response Plan. SOP 1.0, Version 2.0*. USDA, Maryland, USA.

Rift Valley fever

Agent:

Family Bunyaviridae, genus phlebovirus

Agent type:

Virus

Persistence and inactivation:

General characteristics: Rift Valley fever virus is very stable in liquid media (blood or serum) under various conditions of refrigeration, lasting several months at 4°C and three hours at 56°C. It is stable in aerosol at 23°C and 50-85% humidity. RVFV is stable between pH 7-9 but rapidly inactivated below pH 6.8. Virus is inactivated by lipid solvents (ether, sodium deoxycholate and chloroform) and low concentrations of formalin or calcium hypochlorite (residual chlorine should exceed 5000 ppm) (Swanepoel & Coetzer 1994, OIE 2016)

RVF is transmitted by several mosquito species, most notably culicines (Geering *et al* 1995).

Carcases and meat products: There are many reports of humans, such as abattoir workers, becoming infected with RVF after contact with infected tissues. No outbreaks have occurred in urban consumer populations. It is generally considered that meat from RVF-infected animals is not a source of transmission, as the pH changes associated with rigor mortis inactivate the virus (Chambers and Swanepoel 1980, MacDiarmid and Thompson 1997, Swanepoel 1981). The OIE were recently advised of this position (Gerdes, pers comm).

AQIS (1999a) does not consider RVF to be a risk in imported meat products. MacDiarmid and Thompson (1997) concluded that sheep and goat meat presented little risk for the international spread of RVF.

Milk and milk products: RVFV has been detected in low concentration in milk. Infected milk may have been connected with an outbreak of RVF in humans in Mauritania (Swanepoel and Coetzer 1994). DPIE (1996) states that pasteurisation or treatment with acid inactivates the virus. AQIS (1999b) does not regard milk products as posing a threat for the importation of RVF.

Skins, hides and fibres: No specific references were found on this aspect of the disease. In a draft assessment, AFFA (2001) has determined that skins and hides do not pose an import risk for RVF. AUSVETPLAN (DPIE 1996) notes that little is known about the persistence of RVFV in these products but that there is some small risk. It advocates the burial or disinfection of skins and scouring (+/- carbonisation) of fibres.

Semen/embryos: No specific references were found on this aspect of the disease. Radostits *et al* (2000) state that "other than milk and aborted fetuses no body secretions or excretions contain the virus". However, DPIE (1996) notes that RVFV is likely to be present in semen and that transmission is possible by this means, and that the virus is known to be present on ova but is probably not transmitted.

Faeces: Similar comments apply to those regarding skins, hides and fibres. No specific references were found.

References:

AFFA (Department of Agriculture Fisheries and Forestry – Australia) 2001, *Import risk analysis. Skins and hides. Draft report, August 2001*, AFFA, Canberra.

AQIS (Australian Quarantine and Inspection Service) 1999a, *Importation of sausage casings into Australia, import risk analysis, December 1999*, AQIS, Canberra.

AQIS (Australian Quarantine and Inspection Service) 1999b, *Import risk analysis: Importation of dairy products for human consumption*, AQIS, Canberra.

Chambers, P.G., & Swanepoel, R. 1980, 'Rift Valley fever in abattoir workers', *Cent Afr J Med*, vol. 26, pp. 122-126, cited in Swanepoel and Coetzer 1994.

Animal Health Australia, 2013, 'Disease strategy: Rift Valley fever (Version 3.0)' In: *Australian Veterinary Emergency Plan (AUSVETPLAN), Edition 3*, Primary Industries Ministerial Council, Canberra, ACT.

Geering, W.A., Forman, A.J., & Nunn, M.J. 1995, *Exotic diseases of animals: a field guide for Australian veterinarians*, Australian Government Publishing Service, Canberra.

MacDiarmid, S.C., & Thompson, E.J. 1997, 'The potential risks to animal health from imported sheep and goat meat', *Rev sci tech Off int Epiz*, vol. 16(1), pp.45-56.

OIE, 2016, Technical Disease Cards: OIE - World Organisation for Animal Health. Available at: <http://www.oie.int/animal-health-in-the-world/technical-disease-cards/> (Accessed: 15 November 2016).

Paweska J.T, 2015, 'Rift Valley Fever', *Rev. Sci. Tech. Off. Int. Epiz.* Vol 34 (2), pp. 375-389

Radostits, O.M., Gay, C.G., Blood, D.C., & Hinchcliff, K.W. (eds) 2000, *Veterinary medicine*, ninth edition, W. B. Saunders Company Ltd, London.

Swanepoel, R. 1981, 'Observations on Rift Valley fever in Zimbabwe', *Contributions to Epidemiology and Biostatistics*, vol. 3, pp. 83-91, cited in Swanepoel and Coetzer 1994.

Swanepoel, R., & Coetzer, J.A.W. 1994, 'Rift Valley fever', in *Infectious diseases of livestock, Vol I*, eds. Coetzer, J.A.W., Thomson, G.R., & Tustin, R.C., Oxford University Press, Cape Town, pp. 688-717.

Coronaviridae

Porcine Epidemic Diarrhoea Virus

Agent:

Family Coronaviridae

Agent type:

Virus

Persistence and inactivation:General characteristics:

Porcine Epidemic Diarrhoea Virus (PEDV) causes diarrhea, vomiting and high mortality in suckling pigs. Pigs are the only known host of PED.

The faecal - oral route (direct or indirect) is the major route of infection, however Bowman et al (2015) suggested that the disease can be spread by contaminated feed. Pujols et al (2014) state that spray-dried plasma (SDP) proteins are used extensively in nursery pig feed to enhance feed intake, growth, and feed efficiency during the post-weaning period. This feed additive has been suspected of being a potential source of spread of PEDV worldwide, however multiple experimental studies suggest that good manufacturing practices and biosecurity measures make this unlikely (OIE 2016, Pujols et al 2014, Gerber et al 2014).

The ability of the virus to aerosolize and be transported over large distances through air is being considered as an additional important route of virus transmission (Alonso et al 2014).

The OIE states that the virus can survive for variable periods outside the host depending on the temperature and relative humidity. It can survive at least 28 days in slurry at 4°C, 7 days in faeces-contaminated dry feed at 25°C, up to 14 days at 25°C in wet feed and at least 28 days in wet feed mixture at 25°C. The virus loses infectivity above 60 °C and is stable at pH 6.5-7.5 at 37°C and pH 5-9 at 4°C. PEDV is susceptible to; formalin (1%), anhydrous sodium carbonate (4%), lipid solvents, iodophores in phosphoric acid (1%) and sodium hydroxide (2%) (OIE, 2016).

Cha et al (2015) assessed the efficacy of Baroclin®, (citric acid (20% w/v), benzalkonium chloride (10% w/v), and phosphoric acid (6% w/v)) against PEDV. In hard water conditions, PEDV was inactivated by 600-fold dilutions of the disinfectant. In the presence of organic material, the disinfectant showed viricidal activity after a 200-fold dilution. Limitations of this study include the fact that it was only performed in-vitro and that the organic material used may not represent all possible parameters of PEDV contaminated-environments.

Trudeau et al (2016) demonstrated that the risk of spread through contaminated feed can be reduced by heating swine feed at temperatures over 130°C for up to 30 mins or eBeam irradiation processing of feed with a dose over 50 kGy. Additionally, the inclusion of selected additives can decrease PEDV survivability.

Carcases and meat products:

Generic Import Risk Analysis (IRA) for Uncooked Pig Meat (AFFA 2004) states that no studies were identified in which PEDV was demonstrated in muscle tissue, muscle

vasculature, adipose tissues, lymphatic system or the skeletal system. The virus has been identified only in the villous epithelial cells of the small intestine but not in the caecum or colon of naturally infected piglets.

Skins, hides and fibres: No specific reports were found on this aspect of PEDV.

Semen/embryos: There are no reports of PEDV in the semen of boars, or spread of the disease via artificial insemination. As the virus is excreted in the faeces, some risk of semen contamination from this source cannot be excluded.

Faeces:

Thomas et al (2015) demonstrated that PEDV in the presence of faeces on metallic surfaces such as trailers, could be inactivated by heating to 71 °C for 10 minutes or holding at 20 °C for 7 days

References:

AFFA (Department of Agriculture Fisheries and Forestry – Australia) 2004, *Generic import risk analysis (IRA) for uncooked pig meat. Issues paper, February 2004*, AFFA, Canberra.

Alonso, C., Goede, D.P., Morrison, R.B., Davies, P.R., Rovira, A., Marthaler, D.G. and Torremorell, M., 2014. Evidence of infectivity of airborne porcine epidemic diarrhea virus and detection of airborne viral RNA at long distances from infected herds. *Veterinary research*, 45(1), p.1.

Bowman, A.S., Krogwold, R.A., Price, T., Davis, M. and Moeller, S.J., 2015. Investigating the introduction of porcine epidemic diarrhea virus into an Ohio swine operation. *BMC veterinary research*, 11(1), p.1.

Cha, C.N., Yoo, C.Y., Kim, S. and Lee, H.J., 2015. Virucidal efficacy of a disinfectant solution composed of citric acid, benzalkonium chloride and phosphoric acid against porcine epidemic diarrhea virus. *Journal of the Preventive Veterinary Medicine*, 39(2), pp.38-43.

Coffey, R.D. and Cromwell, G.L., 2001. Reviews-Use of spray-dried animal plasma in diets for weaning pigs. *Pig news and Information*, 22(2), pp.39-48.

Gerber, P.F., Xiao, C.T., Chen, Q., Zhang, J., Halbur, P.G. and Opriessnig, T., 2014. The spray-drying process is sufficient to inactivate infectious porcine epidemic diarrhea virus in plasma. *Veterinary microbiology*, 174(1), pp.86-92.

OIE 2016, Technical Disease Cards: OIE - World Organisation for Animal Health. Available at: <http://www.oie.int/animal-health-in-the-world/technical-disease-cards/> (Accessed: 15 November 2016)

Pujols, J. and Segalés, J., 2014. Survivability of porcine epidemic diarrhea virus (PEDV) in bovine plasma submitted to spray drying processing and held at different time by temperature storage conditions. *Veterinary microbiology*, 174(3), pp.427-432.

Thomas, P.R., Karriker, L.A., Ramirez, A., Zhang, J., Ellingson, J.S., Crawford, K.K., Bates, J.L., Hammen, K.J. and Holtkamp, D.J., 2015. Evaluation of time and temperature sufficient to inactivate porcine epidemic diarrhea virus in swine feces on metal surfaces. *J. Swine Health Production*, 23(2), pp84-90

Trudeau, M.P., Verma, H., Sampedro, F., Urriola, P.E., Shurson, G.C., McKelvey, J., Pillai, S.D. and Goyal, S.M., 2016. Comparison of Thermal and Non-Thermal Processing of Swine Feed and the Use of Selected Feed Additives on Inactivation of Porcine Epidemic Diarrhea Virus (PEDV). *PloS one*, 11(6), p.p. 0158128.

Van Dijk, A.J., Everts, H., Nabuurs, M.J.A., Margry, R.J.C.F. and Beynen, A.C., 2001. Growth performance of weanling pigs fed spray-dried animal plasma: a review. *Livestock Production Science*, 68(2), pp.263-274.

Flaviviridae

Japanese encephalitis

Agent:

Family Flaviviridae, genus flavivirus

Agent type:

Virus

Persistence and inactivation:

General characteristics: Japanese encephalitis virus is extremely fragile and does not survive off the host for more than a few hours (DPIE 1998).

Virus is destroyed by heating for 30 minutes at temperatures above 56°C. The thermal inactivation point (TIP) – the lowest temperature required to provide complete inactivation of virus in liquid within 10 minutes – is 40°C. The virus is inactivated in acid environment of pH 1-3 (virus is stable in alkaline environment of pH 7-9). Virus is inactivated by organic and lipid solvents, common detergents, iodine, phenol iodophors 70% ethanol, 2% glutaraldehyde, 3-8% formaldehyde, 1% sodium hypochlorite. The virus is very labile and does not survive well in the environment. The virus is sensitive to ultraviolet light and gamma irradiation (OIE 2016).

Transmission of JEV is principally by *Culex* spp mosquitoes. Pigs are amplifiers of the virus and the only domestic livestock species to develop viraemia of sufficient titre to infect mosquitoes. Virus can be isolated from CNS tissue, CSF and serum (Ellis *et al* 2000).

Carcases and meat products: No references to this aspect of the disease were found in the literature. AUSVETPLAN (DPIE 1998) stipulates no special precautions for the disposal of carcasses from infected premises.

Milk and milk products: Inapparent infections may occur in species producing milk as a product for human consumption (cattle, sheep, goats) (DPIE 1998). No reports were found in the literature regarding the presence or persistence of JEV in milk.

Skins, hides and fibres: In a draft assessment, AFFA (2001) has concluded that the likelihood of JEV transmission via skins, hides or fibres would be negligible.

Semen/embryos: JE has been experimentally transmitted to gilts in boar semen (Habu *et al* 1977). AQIS (2000) has determined that pig semen poses a moderate quarantine risk for JEV.

Faeces: No specific reports were found of the presence or persistence of JEV in faeces.

References:

AFFA (Department of Agriculture Fisheries and Forestry – Australia) 2001, Import risk analysis. Skins and hides. Draft report, August 2001, AFFA, Canberra.

AQIS (Australian Quarantine and Inspection Service) 2000, Draft porcine semen risk analysis: risk assessment and risk management options, March 2000, AQIS, Canberra.

DPIE (Department of Primary Industries and Energy) 1998, Australian Veterinary Emergency Plan, AUSVETPLAN Edition 2.0, Disease Strategy Japanese encephalitis, DPIE, Canberra.

Ellis, P.M., Daniels, P.W., & Banks, D.J. 2000, 'Japanese encephalitis', *Vet Clin Nth Am Eq Pract*, vol. 16, pp. 565-578.

Habu, A., Murakami, Y., Ogasa, A., & Fujisaka, Y. 1997, 'Disorder of spermatogenesis and viral discharge into semen in boars affected with Japanese encephalitis virus', *Virus (Tokyo)*, vol. 27, pp. 21-26, cited in AQIS 2000.

OIE 2016, Technical Disease Cards: OIE - World Organisation for Animal Health. Available at: <http://www.oie.int/animal-health-in-the-world/technical-disease-cards/> (Accessed: 15 November 2016)

Tickborne encephalitides

Agent:

Family Flaviviridae, genus flavivirus

Agent type:

Virus

Persistence and inactivation:

General characteristics: Tickborne encephalitis complex viruses are not very resistant to environmental influences. Tickborne flaviviruses are reported to be sensitive to pH below 6.0 (compared to mosquito-borne flaviviruses, which are sensitive below pH 8.0). They are inactivated by heating to 56°C for 30 minutes but are stable at temperatures below -60°C.

TBE complex viruses are inactivated by UV and gamma radiation and by proteases, lipid solvents and detergents, as well as by low concentrations of aldehydes, halogens, hydrogen peroxide, and beta-propiolactone (Gresikova and Kaluzova 1997, Swanepoel 1994, Reid pers comm, Gould pers comm).

Carcases and meat products: No published reports were found on this aspect of the disease. Gould (pers comm) believes that louping-ill virus would lose its infectivity rapidly (within a day or two) due to putrefaction and bacteria. Inactivation would be slowed by low temperatures. Some virus could be washed out of the carcass but it is likely to be quickly inactivated (days, or probably hours) and diluted below risk level. Similar advice was offered by Reid (pers comm).

Ixodes ticks are normally responsible for transmission. However, there is a report in which 10 out of 16 piglets appear to have become infected through ingestion of the virus (Bannatyne *et al* 1980). Two to three weeks previously, the piglets had been fed the uncooked carcasses of lambs that had died with signs of louping-ill. Grouse chicks have also been infected with louping-ill virus after ingesting infected ticks (Gould pers comm). Although a minor aspect of the epidemiology, these findings would suggest the preferential burial of carcasses rather than leaving in situ.

Milk and milk products: Transmission of louping-ill virus from goat dam to kid via milk has been demonstrated by Reid *et al* (1984). The finding suggested a public health risk consistent with known European cases of human infection by other members of the TBE complex from drinking goat's milk. Outbreaks of disease have also been linked to the milk of infected sheep (Gresikova *et al* 1975) and cattle (Jezyrna 1976). The virus is totally inactivated by pasteurisation using "normal" pasteurisation temperature / time treatments (Reid pers comm).

The commonly applied short-time pasteurisation at 72°C for 15 seconds may not guarantee complete inactivation of the virus when there is a high viral load of TBEV in milk. Heat resistance has also been shown to be strain dependent so broad generalisations may not apply (Saier 2015).

Skins, hides and fibres: The risk of infective TBE complex virus on skins, hides or fibres is negligible, given its relative fragility and normal mode of transmission via ticks (AFFA 2001).

Semen/embryos: No reports of TBE complex virus in semen or embryos were found during this review, although Papadopoulos *et al* (1971) reported the isolation of a TBE complex virus from a flock of goats with abortions. AQIS (2000) concluded that “the risk estimate for [loupings-ill and related viruses] without risk management measures is negligible for semen and embryos”.

Faeces: No references were found on the presence or persistence of TBE complex virus in faeces during this review.

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Wesselsbron disease

Agent:

Family Flaviviridae, genus flavivirus

Agent type:

Virus

Persistence and inactivation:

General characteristics: According to Swanepoel and Coetzer (1994), Wesselsbron disease virus has not been well characterised, but it has the properties typical of haemagglutinating flaviviruses. These are; sensitivity to acidity (< pH 8.0), temperatures above 40°C, lipid solvents and detergents (Gresikova and Kaluzova 1997, Reid pers comm, Gould pers comm).

Transmission of the disease is principally by *Aedes* spp mosquitoes. Handling of infected carcasses has caused infection in humans, and aerosol transmission may be possible. It appears there is no direct spread between sheep (Swanepoel and Coetzer 1994).

Carcasses and meat products: No published reports were found on this aspect of the disease. Humans have been reported to have contracted the disease after performing post mortems on infected animals (Heymann *et al* 1958).

Milk and milk products: No reports were found on the detection or transmission of WDV in milk. AQIS (1999) has listed WDV as an agent that may be excreted in milk, but is not considered a quarantine hazard in milk or milk products.

Skins, hides and fibres: The risk of infective WDV on skins, hides or fibres is negligible, given its relative fragility and normal mode of transmission (AFFA 2001).

Semen/embryos: No reports were found on the detection or transmission of WDV in semen or embryos.

Faeces: No reports were found on the detection or transmission of WDV in faeces.

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Orthomyxiviridae

Avian influenza

Agent:

Family Orthomyxiviridae, type A

Agent type:

Virus

Persistence and inactivation:

Highly pathogenic avian influenza (HPAI) is highly contagious. It is excreted in high concentrations in faeces, and in oral and nasal discharges. Spread is by direct contact, by movement of infected birds, and by fomites (Geering *et al* 1995). The virus is found in the brain, skin, and most visceral organs (Swayne and Suarez 2000).

The wide variation in heat lability between strains of the virus has been noted by Blaha (1989), who reported survival of between 15 minutes and six hours at 56°C. OIE report the virus is inactivated by heat (56-60°C for 60 minutes), inactivated by extremes of pH (1-3 or 10-14) and by ionizing radiation (OIE 2016). Sharhid found that the virus (H5N1) lost infectivity after 30 minutes at 56°C, after 1 day at 28°C, but survived more than 100 days at 4°C in a laboratory experiment (Sharhid *et al* 2009). OIE state that HPAI survival at different temperatures varies ranging from less than 1 day to 7 days at temperatures between 15–35°C under controlled lab conditions (OIE 2016).

HPAI virus survives well in organic matter and in water. It has been isolated from water contaminated with faeces, for up to four days at 22°C and for more than 30 days at 0°C (Webster *et al* 1978). Stallknecht *et al* (1990) estimated retention of infectivity for up to 207 days at 17°C and 102 days at 28°C in lake water. Virus can survive on surfaces for between 2 days to more than 2 weeks (possibly months) if protected from sunlight (OIE 2016). Virus can survive in dust for 2 weeks and for up to 5 weeks in affected poultry houses (Ausvetplan, 2011).

HPAI virus is destroyed by lipid solvents such as detergents, as well as; formalin, sodium hypochlorite, 60-95% ethanol, quaternary ammonium compounds, aldehydes, phenols, acids, povidone-iodine (OIE, 2016). Povidone-iodine products were proven to be effective at inactivating the H5N1 strain of HPAI (Ito *et al.*, 2012). The effectiveness of disinfectants can be affected by organic matter and by the porosity of the surface. All surfaces or items for disinfection should be thoroughly cleaned beforehand (Ausvetplan 2011).

A 2014 study of HPAI (H5N1) in Egypt looked at 4 commercial disinfectants (formalin, Virkon S, TH4 and glutaraldehyde) and concluded that: at low concentrations (0.5 %) in the presence of organic material and on wood, none of these disinfectants were effective at virus inactivation and that higher concentrations (up to 1-2%) and longer contact times (of up to 1 hour) were required to inactivate the virus (Marzouk *et al* 2014).

HPAI virus (H5N1) persistence on glass, wood, galvanized metal and topsoil under changing temperature, humidity and sunlight was studied. Virus was most persistent at low humidity, surviving at least 13 days, and researchers predicted virus may survive up to 2 months in low temperature/low humidity environments (Wood *et al.*, 2010). Exposure to UV light was shown to reduce persistence but its effectiveness was reduced when virus was protected by

soil, chicken faeces and other materials. Sutton et al 2013 concluded that exposure to UV light increased the loss of infectivity. The time taken for a reduction in titre of 1 log median tissue culture infection doses for HPAI H7N1 – 158 minutes and H5N1 -167 minutes. Wood et al 2012 also found that UV light diminished infectivity.

Carcases and meat products:

Kinetic studies on persistence of virus in infected carcasses reveal that tissue (e.g. muscle) could potentially transmit virus for 3 days post mortem whilst other tissues; skin, feather and brain retained infectivity for 5-6 days at 22-23°C (Busquets N et al 2010).

Swayne and Suarez (2000) note, about the risk of importing HPAI in meat products, that “HPAI is a systemic disease and the virus can be present in most tissues, including meat”. AFFA (2001) has determined that there is sufficient prima facie evidence to justify a full risk assessment on HPAI in uncooked chicken meat. The report cites Becker and Uys (1967), who isolated the virus up to six days post-inoculation from the muscle of experimentally infected chickens.

Ausvetplan (2011) states that the virus survives several days in carcasses at ambient temperatures and up to 23 days when refrigerated. It cites the following minimum core temperatures to kill HPAIV:

- 70°C for 30 minutes
- 75°C for 5 minutes
- 80°C for 1 minute.

Senne *et al* (1994) found evidence that composting may be effective in inactivating HPAIV in carcasses. The composting was performed in bins at an ambient temperature of 22°C. Various organs of experimentally-infected birds were composted with poultry carcasses in goat manure and straw in a volume ratio of 1:2:1. The samples were composted in two stages of ten days each, with a turning of the pile at 10 days. The upper layer of the compost reached 57.3°C during the first stage and 41.5°C during the second. Corresponding peak temperatures for the lower layer were 58.3°C and 42.8°C. HPAIV was not isolated at the end of ten days or at twenty days.

Eggs and egg products:

Avian Influenza virus can contaminate albumin, yolk and shell. Egg fillers can also be contaminated. Eggs and fillers can be sanitised with 50-100ppm chlorine (Ausvetplan 2011). Egg pulp products can be contaminated with infective virus. The OIE Terrestrial code provides recommendations for inactivation of Avian Influenza in eggs and egg products. These recommendations are as follows.

- Whole egg - 60°C for 188seconds
- Whole egg blends – 60°C for 188 seconds
- Liquid egg white 55.6°C for 256 seconds
- 10% salted egg 62.2°C for 138 seconds
- Dried egg white 67°C for 0.83 days
- Dried egg white 54°C for 21.38 days (Ausvetplan)

Vertical transmission of HPAI through embryonated eggs has not been demonstrated (Geering *et al* 1995). However, the virus has been detected in and on eggs from naturally infected chickens, arising from deposition during egg formation, and as an external contaminant (Capucci *et al* 1985). No references were found on the persistence of the virus in eggs. Several approaches effectively inactivate AIV in eggs (King 1991). Ackland *et al*

(1985) heated egg waste from influenza vaccine production at 60°C for three hours. King (1991) examined stability of HPAIV at pasteurisation temperatures. Virus was diluted 10⁻² in egg yolk, albumen, or allantoic fluid. HPAIV was inactivated in yolk at 57°C and in allantoic fluid at 62°C in less than 5 minutes. Virus in albumen was inactivated in 5-10 minutes at 57°C.

The author noted current (1991) US recommended high-temperature, short-time pasteurisation times: 3.5 minutes at 57°C for albumen, at 60°C for whole egg, and at 61°C for yolk respectively, or whole eggs at 56°C for 35 minutes, 57°C for 15 minutes, or 60°C for 3.5 minutes. He concluded that these combinations would be marginal even for HPAIV (Newcastle disease virus was hardier) and supported recommendations for their increase. DPIE (1996) states that 4.5 minutes at 64°C is thought to kill HPAIV but that 2.5 minutes is insufficient.

In another experiment, heat and chemical effects on allantoic fluid containing virus, with or without chicken serum, were evaluated. Infectivity of HPAIV was eliminated after 60 minutes at 56°C, and after 30 minutes at 60°C. Formalin 0.01% destroyed HPAIV in serum. Beta-propiolactone 0.1% destroyed the virus in both media, and binary ethyleneimine 0.01M was effective after 5 hours (King 1991).

Other products:

Virus can be present in faeces in concentrations as high as 10⁷ infectious particles per gram, and may persist for more than 44 days (Utterback 1984). HPAIV has been recovered from liquid manure 105 days after depopulation. In other reports, the virus retained infectivity in faeces for 35 days at 4°C and for 7 days at 20°C. The virus can survive in faeces 35 days (at least) at 4°C (Ausvetplan 2011).

Kurmi B et al 2013 found that HPAI (H5N1) in faeces survived 18 hours at 42 °C, 24 hours at 37°C, 5 days at 24°C and 8 weeks at 4°C. This time did not vary greatly for wet or dry faeces in this experiment. This work concluded that contrasting observations regarding faeces implies complexity of factors influencing the virus survivability. Organic material, amount of moisture, exposure to sunlight may play a role in virus survivability.

The presence of organic material protects the virus. Manure can be disposed of by burial, composting in a pile covered by plastic, or rototilling¹ (Easterday *et al* 1997). (Elving J et al 2012) found that rapid inactivation of HPAI (H7N1) was achieved at 35°C and at 45-55°C when composting litter containing faeces. Recommendations included not turning litter material in the first stage of composting and concluded that surface temperature should be kept above 35°C for at least 7.6 hours.

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¹ A definition for this word could not be found

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Equine influenza

Agent:

Family Orthomyxviridae, type A

Agent type:

Virus

Persistence and inactivation:

General characteristics: Equine influenza virus is enveloped and therefore does not survive long outside the host (Mumford 1994). DPIE (1996) quotes persistence of 8-36 hours in the environment.

The properties of equine influenza virus have been described by Yadav *et al* (1993). The virus studied in that report was inactivated by exposure to UV light for 30 minutes or by heating at 50°C for 30 minutes. It was quickly inactivated by; savlon, dettol, phenyl, alcohol, formalin, and potassium permanganate but not by sodium carbonate. (AUSVETPLAN 3.1 2011) advocates 4% lysol. Antec Virkon© showed consistent virucidal efficiency against EIV regardless of reaction time, temperature or presence of organic material (Yamanaka 2014). EIV persisted for up to 14 days in tap water at 4°C or 2 days at 37°C, and in canal water (where colloids were thought to be protective) for 1-2 weeks at 22°C or 37°C. It survived in urine at pH 8.0 for up to 5 days and in soil at 18°C for one day. The virus can survive for 18 hours in horse blood at 37°C (Yadav, 1993).

Influenza viruses are protected by organic matter, which increases resistance to physical and chemical inactivation. Organic material should be removed so disinfectant can work optimally (Swayne and Halosson 2003).

EIV is spread via the respiratory route, almost exclusively, by direct contact between horses. In susceptible populations infection can spread rapidly between premises and over long distances by movement of infected horses. In the 2007 Australian outbreak before imposition of a standstill, infected horses were moved 800 km and introduced disease. Subclinical infection in vaccinated partly immune horses may result in disease spread within endemic areas and internationally. These horses do not become persistent shedders. The virus does not persist in recovered horses. (21-day quarantine after onset of clinical signs should prevent disease spread).

Indirect spread by people and fomites may play some role in disease spread. Mechanical transfer of EI on people, clothes, equipment is thought to have contributed to spread of disease in the 2007 Australian outbreak. Soap and water or alcohol based hand wash applied for 20 seconds are suitable for personal disinfection (Firestone 2011).

Carcases and meat products: No reports on the persistence of equine influenza in horse carcasses were uncovered during this review.

Skins, hides and fibres: EIV from infected aerosols might be expected to superficially contaminate skins, but the unstable nature of the virus in the presence of UV light and heat means that persistence for any period is highly unlikely. This assessment is confirmed by AFFA (2001).

Semen/embryos: EIV is present in the semen and embryos of infected horses, although transmission is unlikely to occur by this route (DPIE 1996).

Faeces: No reports of EIV in faeces were found during this review. Faeces might be expected to be contaminated by aerosols from infected animals. An outbreak of EI in China in 1987 was also distinguished by signs of enteritis in affected animals (Hannant and Mumford 1996).

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Swine influenza

Agent:

Family Orthomyxiviridae, type A

Agent type:

Virus

Persistence and inactivation:

General characteristics: Swine influenza virus (SIV) survival in the environment is influenced by temperature, pH, salinity, and the presence of organic matter. Some viral strains can survive for prolonged periods in low temperatures. Mammalian influenza viruses are relatively labile but can persist for several hours in dried mucous.

SIV is inactivated by heat (56°C for 60 minutes – or a higher temp for a shorter time). Virus is inactivated by ionizing radiation and by low pH (2). SIV is susceptible to a wide range of disinfectants including sodium hypochlorite, 70% ethanol, oxidising agents, quaternary ammonium compounds, aldehydes, phenols, acids, povidine-iodine and lipid solvents (OIE 2016).

The virus shows rapid inactivation in ambient air with approximately 60% inactivation in the first 30 minutes and total inactivation across the next 60-minute period (Oleg 2012).

SIV is transmitted between pigs via the respiratory route. There is no viraemia associated with infection, the virus localising to respiratory and lymphoid tissues (Bachmann 1989).

Carcases and meat products: No reports of the presence of virus in meat from natural infection were uncovered by this review, or by AFFA (2001a). Brown (pers comm) confirmed the lack of data. The only reference of relevance is by Romjin *et al* (1989), who reported a study using pigs inoculated with the H1N1 strain of SIV. Virus was recovered from lung stored at -20°C for between 21 and 28 days, and from blood for at least 14 days (when testing ceased). The report also mentions sporadic recovery of virus from intestine, muscle and faeces.

The authors also examined the survival of virus on artificially contaminated samples of pig meat, from which they could isolate virus for between 8 and 15 days at -20°C. Loss of infectivity was slightly slower at 4°C compared to -20°C.

Skins, hides and fibres: SIV from infected aerosols might be expected to superficially contaminate skins, but the unstable nature of the virus in the presence of UV light and heat means that persistence for any period is highly unlikely. This assessment is confirmed by AFFA (2001b).

Semen/embryos: This review did not uncover any reports of SIV in semen, confirming the finding of AQIS (2000).

Faeces: No reports were found of SIV excretion in faeces. Thomson (1994) cites a study by Kawaoka *et al* (1987), in which only a human strain of influenza virus could be isolated from the faeces of pigs, with two strains of SIV showing no evidence of replication in the intestinal tract.

Bøtner (2012) found that time to inactivation for SIV in slurry varied between 9 weeks at 5°C to 15 days at 20 °C to one hour at 50°C.

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Paramyxoviridae

Hendra virus

Agent:

Family Paramyxoviridae, genus megamyxovirus (proposed name)

Agent type:

Virus

Persistence and inactivation:

Very little information is available on this virus. In experiments involving fruit bats, horses and cats, the virus appeared to be poorly transmissible (Williamson *et al* 1998). The authors concluded that respiratory spread or excretion via faeces was unlikely, and that the most plausible theory for transmission was oral ingestion of urine-contaminated surfaces, water or feed. However, the virus did not appear to persist long in the environment.

CSIRO at AAHL have assumed Hendra virus to have similar properties to Newcastle disease virus until proven otherwise (Daniels pers comm). Virus numbers were reduced by about 6 logs by pure methanol, and by 3% lysol after 3 minutes at room temperature. Anecdotally, the virus appears stable when left in culture at room temperature (Abraham, pers comm).

Hendra is most likely transmitted from horse to horse or horse to humans by contact with infected body fluid or tissue or droplet transmission.

Hendra virus is a lipid envelope virus susceptible outside the host to desiccation and changes in temperature (Fogarty 2008). Experimental evidence suggests survival in the environment varies from several hours to several days depending on environmental conditions (Fogarty 2008). Hendra virus survived days at 22°C in flying fox urine (pH-7). At 37°C the virus was mostly inactivated in less than 1 day. Virus survival on mango depends in part on the acidity of the flesh ranging from 0.3 hours at pH 3 to 22 hours at Ph 5. The virus was rapidly deactivated by desiccation (less than 15 minutes at 37°C) At 22°C virus levels decreased by more than 3 logs within 30 minutes (Fogarty 2008).

Martin et al (2015) found the amount of virus surviving 24 hours ranged from 2% in summer and 12% in winter in northern-most locations (Cairns) and 10% in summer and 33% in winter in southern-most locations (Kempsey). This is significantly shorter than previously assumed. Half-lives of 268 hours, 2.9 hours and 3.5 seconds at temperatures of 4°C, 22°C and 56°C respectively were estimated from modelling (Martin et al 2015). Five days is the presumed maximum survival time for the hendra virus under optimum conditions with a neutral pH, moist air and moderate temperature (Animal Health Australia, 2013).

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Menangle virus (porcine paramyxovirus)

Agent:

Family Paramyxoviridae, genus rubulovirus

Agent type:

Virus

Persistence and inactivation:

Very little experimental work has been conducted on this virus. It exists in only three laboratories: EMAI, AAHL, and CDC (Atlanta). A reasonable assumption is that it has similar properties to Newcastle disease virus, although limited field experience suggests MV may be more fragile in the environment. Sentinel eight-week-old pigs introduced seven days after depopulation failed to contract the disease.

The mode of transmission between pigs is unknown, but infection from faecal or urinary contamination is believed more likely than by respiratory aerosols. There is no evidence of a persistent shedding of the virus by infected animals. In the only cases of Menangle virus reported to date, eradication was achieved using serological testing and segregation.

The virus is rapidly inactivated at low pH (Kirkland pers comm, Kirkland *et al* 2001).

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Newcastle disease

Agent:

Family Paramyxoviridae, genus paramyxovirus

Agent type:

Virus

Persistence and inactivation:

General characteristics: Newcastle disease virus survives well in the environment. NDV may persist for months at 8°C and for several days at 37°C (Beard and Hanson 1984).

NDV is readily inactivated by heating. Some studies have shown strain variation in heat lability, unrelated to virulence (Arzey 1989). In nutrient broth, inactivation times at 56°C varied between five minutes and six hours. All strains were inactivated at 100°C after 60 seconds (Beard and Hanson 1984). The OIE (2016) regard NDV to be inactivated at temperatures of 56°C for 3 hours or 60°C for 30 minutes. The virus is inactivated by acid (pH < 2) (OIE 2016).

NDV is sensitive to a wide range of disinfectants, including chlorine-based chemicals and lipid solvents (AFFA 2000). NDV is ether sensitive, inactivated by formalin, phenolics, oxidising agents (eg Virkon®), chlorhexidine, sodium hypochlorite (OIE 2016).

Calcinated egg shell (Egg-CaO) as a powder or aqueous solution inactivated Newcastle Disease Virus. This product was shown to be safe for use in litter or feed. Egg-CaO can provide a useful enhancement of biosecurity in ND outbreaks (Mari et al).

Transmission of NDV between birds occurs through inhalation of droplets or ingestion of material such as faeces, with the latter appearing to be the more important in the field (Alexander 1995). Rodents were shown to harbour the virus during one outbreak and were suggested as a mechanism of spread in another (Arzey 1989). Fomites e.g. feed, water, implements, premises, clothing, boots, sacks, egg trays, can transmit NDV.

Virus persist longest in faeces. After depopulation in outbreak control, no Newcastle Disease Virus was found in manure after day 16 post depopulation (Hailu et al).

The persistence and inactivation of NDV in poultry products has been thoroughly reviewed by Arzey (1989).

Carcases and meat products: NDV is found at various times in most organs and tissues, the distribution and concentration dependent on the virulence of the strain and on tissue tropism (Hofstad 1951, Sinha *et al* 1952). Velogenic and mesogenic strains of NDV have been isolated from carcasses at slaughter (Lancaster and Alexander 1975). Gordon *et al* (1948) attributed one third of the first 542 outbreaks of ND in England and Wales to feeding poultry waste to chickens. Reid (1961) isolated NDV from up to two-thirds of frozen poultry imported into Britain that year.

Survival of the virus depends on its initial concentration, the ambient temperature and humidity, and length of exposure (Foster and Thompson 1952, Olesiuk 1951). Reported survival times include:

- 96 days on the skin and 134 days in bone marrow of eviscerated plucked carcasses held at 2°C

- 160 days on the skin and 196 days in bone marrow of eviscerated unplucked carcasses held at 2°C
- greater than 300 days on skin and in bone marrow of both plucked and unplucked eviscerated carcasses held at -20°C (Asplin 1949)
- 270 days on skin and in meat at -14°C to -20°C for a mesogenic strain (Michalov *et al* 1967).

Arzey (1989) discusses the heating requirements for inactivation of NDV in poultry meat products, and the time/temperature combinations achieved in various commercial processes, at some length. He notes that the thermostability data from studies using nutrient broth may not apply in meat because of the protective environment offered by meats. One minute at 75°C is suggested as sufficient for meat with low titre NDV. For waste foods fed to poultry, one minute at 100°C may be required to remove all virus activity.

Wooley *et al* (1981) found that NDV survived for at least 96 hours (the entire test period) in *Lactobacillus*-fermented food waste material held at 5°C, 10°C, 20°C, and 30°C. pH dropped as low as 3.5 at 30°C.

Eggs and egg products: Newcastle disease virus has been isolated from eggs (e.g. Zagar and Pomeroy 1950). Williams and Dillard (1968) found NDV in the membranes below the shell. Dried egg has also been found to contain NDV (Alegren 1951). Eggs are therefore an infective risk both from external faecal contamination and from within the egg.

Arzey (1989) notes that NDV would be better protected against heat in the medium of a liquid egg than it would in nutrient broth, in which the thermostability data of Foster and Thompson (1957) were derived (see above). Gough (1973) isolated NDV from liquid egg that had been heated to 64.4°C for 200 seconds. King (1991) reported that pasteurisation did not destroy NDV in egg, serum or viral diagnostic agents.

Other products: NDV is found in the faeces of infected chickens. It has been reported to persist in uncultivated manure for more than six months (Alexander *et al* 1985), and for 20 days in manure heaped in mounds, where internal temperatures would have been higher (Zakomirdin 1963).

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Nipah virus

Agent:

Family Paramyxoviridae, genus megamyxovirus (proposed name)

Agent type:

Virus

Persistence and inactivation:

Nipah virus is found in urine and uterine fluids of wild pteropid bats and has been experimentally isolated from urine, kidney and uterus of infected bats. The virus may be found in fruit contaminated with bat saliva or urine.

Work at AAHL has shown that pigs can become infected with Nipah virus via either the oral route or by parenteral inoculation, and that the virus is excreted via oronasal routes.

Experience in Malaysia has suggested urine, saliva, pharyngeal and bronchial secretions and semen may be involved in transmission. It is also suspected that dogs and cats may have become infected and/or mechanically transferred the infection from pig trucks (Mohd Nor *et al.*, 2000).

Survival:

Survival of the virus is highly sensitive to desiccation, temperature and pH. Under most conditions survival time is brief with a half-life of only a few hours. Nipah virus has been shown to survive in fruit bat urine (pH of 7) and contaminated fruit juice for more than two days (Fogarty 2008).

Whilst virus in solution has been shown to tolerate extremes of pH (ranging from 3-11), the combination of pH, temperature and substrate composition (e.g. fruit type) strongly influences survival. Outbreaks may be more common during periods where the combination of fruit availability and ripeness, bat feeding activity, bat urine pH and environmental conditions allow longer virus survival outside of a host.

Other paramyxoviruses are readily inactivated after 60°C for 60 minutes. They are also stable at pH 4.0-10.0. Paramyxoviruses are susceptible to common soaps and disinfectants; lipid solvents (alcohol, ether) and sodium hypochlorite. These were used effectively in outbreaks for cleaning and disinfection (OIE 2016).

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Peste des petits ruminants

Agent:

Family Paramyxoviridae, genus morbillivirus

Agent type:

Virus

Persistence and inactivation:

General characteristics: Relatively little work has been done on the persistence of the peste des petits ruminants virus outside the animal, although it is reported to have similar physico-chemical characteristics to rinderpest virus (Rossiter and Taylor 1994). Taylor (1990 unpublished cited in Rossiter and Taylor 1994) reported on a strain of the virus from India² with the following thermal stability:

Temp (°C)	Half-life
-20	24.2 days
4	9.9 days
37	3.3 hours
56	2.2 minutes

The virus is destroyed at temperatures of 50°C for 60 minutes. The virus is inactivated at pH <4.0 or >11.0 (OIE 2016).

Effective disinfectant agents include alcohol, ether, and common detergents. Virus is susceptible to most disinfectants e.g. phenol, sodium hydroxide (OIE 2016).

AUSVETPLAN (Animal Health Australia 2009) recommend that halogens and alkalis are suitable for disinfecting buildings, concrete, structures, equipment. For personal disinfection, citric acid, alcohols and iodophors are suitable. The virus is rapidly inactivated by UV light and desiccation within 4 days (Animal Health Australia 2009).

Lefevre (1987) reported comparable data, estimating a half-life of around two hours at 37°C and inactivation within one hour at 50°C. The virus studied by Taylor was stable between pH 5.85 and 9.5 and rapidly inactivated below pH 4.0 or above pH 11.0. Rossiter and Taylor (1994) advise the disinfection of infected premises with lipid solvents or agents with low or high pH.

PPR is characterised by high viraemia and multiplication of the virus in a wide range of tissues. PPRV is shed in ocular, nasal, and oral secretions and faeces of infected animals. Transmission is mainly by inhalation of aerosols from close contact animals, and by nuzzling and licking. Recently contaminated fomites may also be a source of infection (Rossiter and Taylor 1994).

Carcases and meat products: Viable virus can be recovered for at least eight days from lymph nodes from carcasses stored at 4°C (Lefevre 1987). No other references were found during this review regarding the persistence of PPRV in carcasses or meat products. AUSVETPLAN (Animal Health Australia 2009) recommend that carcasses be hung to allow pH to decrease to

² Geering *et al* note that there is some doubt whether the disease in India is caused by PPRV or rinderpest virus, so these data should be treated with some caution.

level that will destroy PPR virus. Meat should not be chilled quickly. AQIS (1999a) have concluded that the risk of introducing PPRV via sausage casings is probably low.

However, note should be made of several reports of persistence of rinderpest virus. Blackwell (1984) cites recovery of active virus from carcasses stored at 4°C for 30 days, and from carcasses aged for 24 hours and then kept at 4°C for 8 days. The same paper reports recovery of virus from carcasses buried for two months. Ezzatt *et al* (1970) have reported infectivity of meat refrigerated for 7 days. Rinderpest virus may survive for several months in salted meat (Blaha 1989).

Although at least one outbreak of rinderpest has been attributed to the ingestion of fresh meat (Rossiter 1994), the risk of this happening with sheep or goat meat is regarded as low (MacDiarmid and Thompson 1997).

Milk and milk products: No primary references were found on the presence or persistence of PPRV in milk. AUSVETPLAN (Animal Health Australia, 2009) require disposal of all milk and milk products originating from infected premises. Marketing of milk from non-exposed animals on dangerous contact properties (DCPs) can only be after heat treatment of milk to make milk powder. This is because pasteurisation alone may not inactivate PPR virus.

Skins, hides and fibres: The presence of virus on the skin of infected animals, by either excretion or external contamination, is highly likely. Rinderpest virus has been shown to rapidly lose infectivity on adequately dried infected hides (Beaton 1932), and it is reasonable to extrapolate this finding to PPRV. The virus is inactivated by the liming and pickling, so treated and partially treated hides are not considered to pose an infective risk (AFFA 2001). However, salting of skins is probably protective of the virus. In a draft assessment, AFFA (2001) has concluded that unprocessed skins and hides from susceptible animals in countries with PPRV pose a high quarantine risk.

AUSVETPLAN (DPIE 1996a) states that, in the case of an outbreak, skins, hides or fibres deemed to be infected may need to be destroyed.

Semen/embryos: No primary references were found on the presence or persistence of PPRV in semen or on embryos, although DPIE (1996a) states that the virus is present in semen and embryos and is likely to be transmitted by them. The evidence on which this judgement is based is unclear, but infection is clearly a risk given the isolation of virus from other secretions.

Faeces: PPRV may be found in the faeces of infected animals (Rossiter and Taylor 1994). No specific references were found on its persistence. AUSVETPLAN (DPIE 1996a) states that in the event of an outbreak, faeces should be buried.

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Rinderpest

Agent:

Family Paramyxoviridae, genus morbillivirus

Agent type:

Virus

Persistence and inactivation:

General characteristics: Rinderpest virus is a relatively unstable virus. It retains infectivity for only a few hours outside the host, except in very favourable conditions, when it may persist for up to two to four days (Rossiter 1994). The virus shows greatest stability at pH 7.2-7.9, and is rapidly inactivated outside pH range 5.6-9.6 (Geering *et al* 1995). It is killed quickly at temperatures above 70°C (De Boer and Barber 1964) but small fractions of tissue-cultured virus have survived heating at 56°C for 50 to 60 minutes and at 60°C for 30 minutes (Timoney *et al* 1988). Parsonson (1992) has cited further thermal stability data from Scott (1959):

Temp (°C)	Protein content of medium	Time exposure	Initial titre (log ₁₀ TCID ₅₀ /mL)	Titre reduction
-70	5% calf serum	17 weeks		No loss
-25	5% calf serum	23 weeks		No loss
4	5% calf serum	> 8 weeks	Half-life: 9.2 days	
25	0.125%	24 hours	2.14	0.5
37	5% calf serum	48 hours	Half-life: 165 minutes	
56	5% calf serum	60 minutes	Half-life: 3.5 minutes	

Rinderpest virus is sensitive to light and UV radiation and dessication.

In general alkalis, halogen and phenolic compounds are good for disinfecting buildings, floors and equipment. AUSVETPLAN stipulate personal disinfection with either citric acid, alcohol iodophors (Animal Health Australia 2009).

Rinderpest virus is present in the ocular, nasal, oral, and vaginal secretions and faeces of infected animals. It is transmitted by predominantly by direct contact and possibly by aerosols over short distances or even by windborne spread (Rossiter 1994).

Carcases and meat products: Susceptibility to decreases in pH post mortem would suggest that rinderpest virus does not generally survive long in carcasses. Rossiter (1994), citing Curasson (1932), states that the virus is inactivated by carcase decomposition within one to three days. However, Blackwell (1984) reported recovery of active virus from carcasses stored at 4°C for 30 days, and from carcasses aged for 24 hours and then kept at 4°C for 8 days. The same paper reports recovery of virus from carcasses buried for two months. The virus has also been recovered from meat frozen prior to rigor mortis (Blaha 1989). Ezzatt *et al* (1970) have reported infectivity of meat refrigerated for 7 days. Outbreaks of rinderpest have been attributed to the ingestion of fresh meat (Rossiter 1994).

AUSVETPLAN states that virus is rapidly inactivated by autolysis and putrefaction and does not survive more than 24 hours in carcase of animal that has died of the disease (Animal Health Australia 2009).

Rinderpest virus may survive for several months in salted meat (Blaha 1989). No other reports were found during this review or by AFFA (2001a) on the persistence or inactivation of rinderpest virus in meat products.

Milk and milk products: No primary references were found on the presence or persistence of rinderpest virus in milk. Animal Health Australia (2009) and AQIS (1999) cite Plowright (1964) to state that virus can be present in milk from 1-2 days before clinical signs develop and for up to 45 days after recovery. The same information is quoted by Blackwell (1984), citing another review. Later review articles do not mention the presence of rinderpest virus in milk (Rossiter 1994, Timoney *et al* 1988).

If the virus is present in milk, prolonged survival is unlikely and pasteurisation should cause inactivation, but this is unconfirmed (AQIS 1999). AUSVETPLAN therefore stipulate the heat processing of milk into milk powder (only) in milk from restricted and control areas during an outbreak (Animal Health Australia 2009).

Skins, hides and fibres: The presence of virus on the skin of infected animals, by either secretion or external contamination, is highly likely. Infectivity is lost rapidly from adequately dried infected hides (Beaton 1932). The virus is inactivated by the liming and pickling, so treated and partially treated hides are not considered to pose an infective risk AFFA (2001b). However, salting of skins is probably protective of the virus. In a draft assessment, AFFA (2001b) has concluded that unprocessed skins and hides from susceptible animals in countries with rinderpest pose a high quarantine risk.

AUSVETPLAN (DPIE 1996) stipulates disinfection of skins, hides or fibres before removal from restricted and control areas in the case of an outbreak.

Semen/embryos: No primary references were found on the presence or persistence of rinderpest virus in semen or on embryos, although DPIE (1996) refers to “very early work” demonstrating semen transmission. Little virus is excreted via the reproductive tract (Philpott 1993). The virus is found in vaginal discharges of infected cows (Rossiter 1994). AUSVETPLAN regard the risk of transmission via this route to be negligible if embryos are handled according to IETS manual (2004) (Animal Health Australia 2009).

Faeces: Rinderpest virus may be found in the faeces of infected animals (Rossiter 1994). No specific references were found on its persistence.

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Picornaviridae

Foot-and-mouth disease

Agent:

Family Picornaviridae, genus aphthovirus

Agent type:

Virus

Persistence and inactivation:

General characteristics: Foot-and-mouth disease has been thoroughly reviewed by Thomson (1994). FMDV is very labile in acid and alkaline conditions. Donaldson (1987) states that stability is greatest at 7.4-7.6, but with survival at 6.7-9.5 at below 4°C. Bachrach (1968) reported that the time to reduce infectivity by 1 log₁₀ at varying pH was:

pH	Time
5	1 second
6	1 minute
6.5	14 hours
10	14 hours

FMD virus is quickly inactivated by pH <6.0 or >9.0 (OIE 2016).

For differing temperatures, the time to reduce infectivity by 1 log₁₀ was:

Temp	Time
4°C	1 week
20°C	11 days
37°C	21 hours
49°C	1 hour
61°C	3 seconds

The virus is preserved by refrigeration and freezing. It is inactivated at temperature > 50°C. Heating meat to minimum core temperature of 70°C for 30 minutes inactivates the virus (OIE 2016). Woese (1960) and Bengis (1997) note that 69°C “appears to be the critical temperature for inactivation. FMDV is stable almost indefinitely below 4°C (Donaldson 1987). FMDV is resistant to sunlight, but sensitive to drying, with poor survival below a relative humidity of 55-60% (Thomson 1994).

AFFA (2001a) cites other reports of FMDV survival in the environment – for example: 50 days in water, 26-200 days in soil, hay, sacking or straw, 345 days on one farm in California in 1924.

Disinfectants – the virus is inactivated by sodium hydroxide (2%), sodium carbonate (4%), citric acid (0.2%), acetic acid (2%) sodium hyperchlorite (3%) potassium peroxymonosulfate/sodium chloride (1%) and chlorine dioxide. The virus is resistant to iodophors, quaternary ammonium compound and phenol especially in the presence of organic matter (OIE 2016).

For porous surfaces (e.g. wooden floors) citric acid at concentrations of least 2% is recommended (Krug et al 2012).

In viraemic animals, FMDV is found in all physiological fluids and therefore nearly all excretions and secretions. The virus appears for up to four days prior to clinical signs. Infection takes place via oral, respiratory and possibly venereal routes, while transmission is by direct contact, by windborne spread, and by fomites. Different species showing varying susceptibility to these modes (Thomson 1994).

The survival of airborne virus is mainly influenced by relative humidity (RH) with good survival above 60% RH and rapid inactivation below 60% RH (Donaldson 72).

Carcases and meat products: The risk of FMDV infectivity in meat products has been reviewed by Callis (1996), and in pig meat products specifically by AFFA (2001b) and Farez and Morley (1997).

Theoretically there could be airborne spread of Foot and Mouth Disease Virus during the incineration process, but there is yet to be evidence to prove this (Champion et al., 2002; J. Gloster et al., 2001). The OIE states that there may be particulate dissemination due to incomplete combustion via pyre burning (OIE, 2016).

FMDV is distributed throughout the body. Survival of the virus post-mortem depends on the stage of disease at time of slaughter, the strain of virus and environmental factors, especially temperature and hydrogen ion concentration. The pH changes associated with rigor mortis are sufficient to inactivate FMDV in muscle within 24 to 72 hours after slaughter. However, caution in extrapolating findings between species is recommended by Bengis (1997), citing the case of highly myotropic FMDV surviving beyond 72 hours in the carcass of impala, when the pH had dropped to 5.6.

Refrigeration suspends the formation of acid, in which case the virus can survive for weeks or months, especially in lymph nodes, blood clots, bone marrow and viscera (Henderson and Brooksby 1948, Callis 1996). Cottral (1969) reported survival of the virus for 120 days in chilled lymph nodes.

Gomes *et al* (1994) detected the O1 strain of FMDV in semimembranosus and longissimus dorsi muscles and internal organs of sheep that were slaughtered during the febrile state. The muscles did not reach a pH of less than 6. The virus was detectable both before and after maturation of the carcass. It survived four months of freezing at -20°C. Virus was not detectable in sheep slaughtered at 15 or 30 days post-infection.

The OIE International Animal Health Code recommends meat be heated to a core temperature of at least 70°C for 30 minutes, but this may be insufficient to ensure complete inactivation. Mincing generally helps to reduce FMDV survival and lactic-curing of salamis ensures inactivation of any virus within a week. The virus can survive for long periods in high salt concentrations, such as those found on sausage casings. Washing of infected intestines in 0.5% to 2.0% lactic or citric acid, or a citric acid buffer system (pH 5.3), for 8 to 10 hours is recommended (MacDiarmid and Thompson 1997).

FMDV has also been shown to survive for up to:

- 60 days in beef held at 4°C in brine (Cottral *et al* 1960)
- 30 days in chilled pig lungs, stomach, tongue, intestine, 24 hours in chilled pig spleen, liver and kidney, and 210 days in frozen pig organs (Savi *et al* 1962)
- 112 to 190 days in various hams and bacon (Dhenin *et al* 1980, McKercher *et al* 1987, Mebus *et al* 1997)
- 42 days in Iberian pork loins (Mebus *et al* 1997)
- 56 days in pork sausages (Dhenin *et al* 1980)

- 250 days in processed pig intestinal casings (McKercher *et al* 1978)
- 7 days in pork salami (Panina *et al* 1989)
- 10 days in pork tongue and 1 day in muscle (Cottral 1969).

Milk and milk products: The high potential for transmission of FMDV via milk and milk products has been reviewed by AQIS (1999a), Callis (1996) and Donaldson (1997). FMDV may be excreted in milk before clinical signs are apparent and disappears with the development of neutralising antibody (Callis 1996). The virus shows biphasic temperature and pH inactivation curves in which there is an initial rapid phase, and then a more protracted phase of inactivation (Bachrach *et al* 1957). Some virus may survive pasteurisation, probably due to its association with cell debris, although Donaldson (1997) has argued that the risk posed by this small residue of virus, in terms of infecting calves or pigs, is very small. Ultra high temperature (UHT) processing is sufficient to cause complete inactivation (Cunliffe *et al* 1979). Heating of skim and full-cream milk to 80-90°C for 30 seconds has been shown to reduce infectivity by $10^{5.4} - 10^{6.0}$ ID₅₀ (Van Bekkum and de Leeuw 1978). Residual virus survives in milk and milk products following regular (low heat) pasteurisation but is inactivated by ultra high temperature (UHT) pasteurisation (OIE 2016).

FMDV titre is reduced but not eliminated by processing of casein or caseinates, although after 30 days, infectivity could not be demonstrated (Callis 1996). Similarly, FMDV has been shown to survive the processing of certain cheeses (e.g. cheddar made from milk that was not heat-treated, for somewhere between 60 and 120 days at pH 5.0; camembert for one day after processing), but infectivity disappears with ageing and ripening (Donaldson 1997).

In the opinion of Callis (1996), cheese production would be a good option of disposing of milk collected during an outbreak. Milk from infected animals should not be fed back to livestock as milk or other products (caseinate, whey, dry milk powder) unless the milk is UHT-processed.

Skins, hides and fibres: In a draft assessment, AFFA (2001c) has identified hides and skins as major potential hazards for the introduction of FMDV, both through surface contamination and persistence in the skin itself.

FMDV virus has been recovered from dried hides for up to eight days, and from salted hides for up to 352 days at 4°C (Gailiunis and Cottral 1967). The salt appears to be protective of the virus. Sodium carbonate can be added to the salt to raise pH, but whilst the OIE International Animal Health Code stipulates salting for 28 days with 2% added sodium carbonate to inactivate FMDV, AFFA (2001c) points to the lack of information on the pH produced by this mixture. There is also some confusion about whether the 2% is a proportion by weight of the hide or the salt.

The virus is inactivated by the high pH (>12.5) involved in liming and dehairing of skins and by the low pH of pickling and tanning. Fully tanned or fully processed skins and hides (including 'wet whites' and 'wet blues') are therefore considered to pose a negligible risk. Disinfection with acids or alkalis, low concentrations of formaldehyde, or 570g/m³ ethylene oxide gas at 52°C for 160 minutes will also be effective (AFFA 2001c).

FMDV has been isolated from greasy wool for up to 14 days after experimental contamination. The virus survived for 7 weeks at 4°C, for 2 weeks at 18°C, and for 2 days at 37°C (McCcoll *et al* 1995).

Semen/embryos: The presence of FMDV in bull semen and its potential to infect cows during artificial insemination has been demonstrated by Cottral *et al* (1968). FMDV has survived for

a month in frozen semen (Giefloff *et al* 1961). Virus may be shed for up to four days prior to clinical signs, and for up to 42 days afterwards. Virus may also be found in the semen of boars before and after disease (Callis 1996, Sellers 1983). Recently, Bastros *et al* (1999) have demonstrated the presence of FMDV in the semen and sheath of wild buffaloes.

Callis (1996) summarised the work of several authors on the spread of FMDV by embryo transfer, as follows:

- bovine, ovine and caprine embryos with intact zona pellucida were free of virus after exposure to 10^6 plaque-forming units per mL of FMDV then washing according to International Embryo Transfer Society standards;
- similar findings applied to embryos taken from acutely-infected stock and washed to IETS standards;
- susceptible cows implanted with these embryos did not become infected;
- zona-pellucida intact bovine embryos resulting from insemination of a cow 90 days post-infection, with semen from a bull 40 days post-infection, and washed to IETS standards were free of the virus;
- a similar study using sheep and goats resulted in virus-free embryos; but
- embryos without an intact zona pellucida did become infected.

These findings indicate a very low risk of transfer of FMDV by embryo transfer if zona pellucida-intact embryos are used and are handled according to IETS recommendations. AQIS (1999b) and Suttmoller and Wrathall (1997) have reached the same conclusion.

There is little information on swine embryos, although there is some indication that the virus may be more prone to sticking to the zona pellucida than in bovine embryos (Callis 1996).

Faeces: Haas *et al* (1995), in a review of virus survival in liquid manure, quote several studies on the survival of FMDV. Muller (1973) reported survival of FMDV for 21-103 days. Bøtner (1990) reported survival times ranging from >14 weeks at 5°C to 1 hour at 55°C in pig slurry, and between 5 weeks at 20°C and >60 minutes (inactivation not reached) at 55°C in cattle slurry. Initial concentration of virus was $10^{4.8}$ TCID₅₀/50uL in each case. Eizenberger (unpublished, cited in Haas *et al* 1995) demonstrated survival of FMDV in cattle slurry of 84 days at 4°C to 70 days at 17°C. AFFA (2001a) cites several figures for survival in manure, ranging from 6 to 42 days.

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Swine vesicular disease

Agent:

Family Picornaviridae, genus enterovirus

Agent type:

Virus

Persistence and inactivation:

General characteristics: Swine vesicular disease virus is a very hardy agent. It is relatively stable over a pH range of 2-12, and has survived for 164 days at pH 5.10 and pH 7.54 at 5°C. The virus lost 6 logs of infectivity after 164 days at pH 2.88 and pH 10.14, and after 38 days at pH 1.92 and pH 11.96 (Herniman *et al* 1973). SVDV is more resistant to heating and desiccation than FMD virus (Geering *et al* 1995), and can withstand freezing, although it is inactivated at 69°C (Loxam and Hedger 1983).

SVDV is resistant to many common disinfectants and detergents (DPIE 1996). Thomson (1994) recommends the use of an alkali such as 1% sodium hydroxide for disinfection in the presence of faeces or other organic matter.

SVDV is inactivated by sodium hydroxide (1% combined with detergent) – even in the presence of organic matter. Direct treatment of swine waste with 1.5% (w/v) NaOH or CaOH₂ for 30 minutes was found to inactivate SVDV at both 4°C or 22°C. A mixture of didecylmethylammonium chloride and 0.1% NaOH applied for 30-60 minutes also was effective. For personal disinfection and in the absence of gross organic matter, disinfectants, such as oxidising agents, iodophors, acids etc. are suitable if combined with detergents (OIE 2016).

The major source of virus in transmission is ruptured vesicles. SVDV is also excreted in the faeces for 20 days or more, although this represents a less significant source of virus. Spread occurs through direct contact, or indirectly via fomites or swill feeding. Airborne spread is not a feature of the epidemiology. Infection requires a much smaller dose of virus through abraded skin than it does to establish through oral, nasal or ocular routes (Geering *et al* 1995, Thomson 1994).

SVDV is preferentially found in the epithelium of the coronary band, tongue, snout and lips as well as myocardium, tonsils and brain stem (Thomson 1994). McKercher *et al* (1974) have demonstrated transmission of SVD to pigs fed infected meat.

Carcases and meat products: The survival of SVDV in carcasses and meat products has been comprehensively reviewed by Farez and Morley (1997). SVDV is resistant to the pH changes accompanying rigor mortis (AFFA 2001a). It is stable in infected tissue kept at ambient or higher temperatures for at least four months (Thomson 1994). Dawe (1974) reported that there was no loss in infectivity of SVDV, 11 months after slaughter, in carcasses frozen at -20°C. The skin yielded 10⁶ TCID (tissue culture infective doses) per gram, intercostal muscle 10⁴ TCID, and rib bone and kidney cortex 10³ TCID, like titres detected at the beginning of the storage period. Watson (1981) reported no drop in titre of SVDV in carcass material held for twelve months at 12-17°C.

The potential risks associated with biomal (fuel production from animal waste) in Europe and spread of SVDV have been examined. Vinneras *et al.* (2012) concluded that transmission of SVDV could occur following leakage from biomal. The virus persisted up to 168 days at low temperature – even after the addition of formic acid to the ground animal carcasses. However,

Sahlstrom (2008) found that heat treatment at 70°C for 30 minutes in the process of biogas manufacture was sufficient to inactivate SVDV.

Several studies have looked at the persistence of SVDV in meat products, for example:

- up to 14 to 84 days in Iberian dry-cured loins and shoulders (within the commercial curing period) (Mebus *et al* 1997)
- up to 470 days in Iberian and Serrano dry-cured hams (exceeding the commercial curing period for Serrano ham) (Mebus *et al* 1997)
- at least six months in Parma hams (McKercher *et al* 1978)
- 200 days in dry salami, dry pepperoni sausage and intestinal casings (McKercher *et al* 1974)
- a minimum of 780 days in intestinal casings in another study (McKercher *et al* 1978).

Farez and Morley (1997) stated that “apparent thermal inactivation of SVD virus is obtained by heating to at least 69°C”.

SVDV survives for many months in buried carcasses (DPIE 1996).

Skins, hides and fibres: There is little primary information available on the persistence of SVDV on skins. In a draft assessment, AFFA (2001b) has concluded that there is unlikely to be many virus particles within the skin of an infected animal unless it is prepared early in an outbreak. Given its hardy nature, however, there is a high likelihood that the virus would survive on skins and hides during transport. Unprocessed or partially processed skins could not be relied on to be free of virus. Only fully processed or fully tanned skins (including ‘wet whites’ and ‘wet blues’) are considered to pose no quarantine threat.

Semen/embryos: SVDV has been found in the semen of infected boars from 2-3 days after infection and 1-2 days before clinical signs. Attempts to establish infection in gilts by artificial insemination with infected semen were generally unsuccessful. One pig receiving a large dose of virus via the semen (10^8 pfu) did become infected, but so too did another pig in the room at the time (McVicar *et al* 1977).

In the absence of other evidence, AQIS (2000) has concluded that transmission of SVDV via artificial insemination is unlikely. Similarly, DPIE (1996) notes that transmission by infected embryos is unlikely if proper procedures are followed.

Faeces: Dawe (1974) recovered SVDV from faeces for up to 138 days in ambient temperatures of 12-17°C. The temperature subsequently rose to 25°C and the virus was not recovered.

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Teschen disease (porcine polioencephalomyelitis)

Agent:

Family Picornaviridae, genus enterovirus

Agent type:

Virus

Persistence and inactivation:

General characteristics: Teschen disease virus is a relatively stable agent. Porcine enteroviruses can survive more than 168 days in the environment at 15°C (Leman *et al* 1986). Derbyshire (1989) stated that Teschen disease virus can remain infective for 15 minutes at 60°C and for longer periods at 56°C, while Idriss (1983) reported inactivation of porcine enteroviruses by 8 weeks at 22°C and after 15-25 minutes at 56°C.

TDV survived for more than 168 days in water at 9-15°C, with chlorine at 0.1mg/L producing a 1 log reduction within two hours (Ottis 1976). TDV is stable between pH 2.8 and 9.5 but rapidly inactivated outside this range (Derbyshire 1989). In a study involving ten common disinfectants, only sodium hypochlorite and ethyl alcohol completely inactivated the virus (Derbyshire and Arkell 1989).

TDV is spread via the oral-faecal route and probably also by fomites (Derbyshire 1989).

Carcases and meat products: No specific reports on the persistence or inactivation of TDV in carcasses or meat products were identified by this review, or in an issues paper by AFFA (2001a).

Skins, hides and fibres: AFFA (2001b), in a draft analysis, have concluded that processing or partial processing of pig skins “should effectively eliminate the risk with porcine enteroviruses”. Teschen disease virus is unlikely to survive the preliminary scalding of slaughtered pigs, or the alkaline scouring, acid pickling or liming / dehairing of skins. Unprocessed skins would pose a threat because of the stability of the agent in the environment.

Semen/embryos: Porcine enteroviruses have been found in semen (Phillips *et al* 1972). While TDV is likely to be present in semen during the viraemic stage, transmission via artificial insemination is considered unlikely as viruses “do not productively infect early embryos” (Thomson 1994).

Faeces: Derbyshire (1989) summarised studies showing that inactivation of the virus is more rapid in aerated slurry – for example, 1 log decrease at 20°C in 2-4 days (aerated) vs 300 days at 5°C (un-aerated). One strain of porcine enterovirus (T80 of serotype 2) was inactivated in pig slurry by treatment with calcium hydroxide at pH 11.5 (Albrecht and Strauch 1980; Derbyshire and Brown 1979; Lund and Nissen 1983).

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Poxviridae

Lumpy skin disease

Agent:

Family Poxviridae, genus capripoxvirus

Agent type:

Virus

Persistence and inactivation:

General characteristics: Lumpy skin disease (LSD) virus is a stable agent, remaining viable in scab or tissue for long periods. It is stable between pH 6.6 and 8.6. It has persisted for 5 days at 37°C in this pH range without significant loss of titre (although AFFA (2001) note conflicting reports on heat stability). LSDV has shown infectivity in dried skin lesions on the animal for at least 33 days, and 18 days in scrapings from dry lesions at room temperature (Weiss 1968). The virus is susceptible to sunlight but in dark conditions (such as contaminated animal sheds), it can persist many months. Virus is susceptible to heat with inactivation at 55°C in 2 hours, and at 65°C in 30 minutes. Virus can be recovered from skin nodules kept at -80°C for 10 years and in infected tissue culture fluid stored at 4°C for 6 months. (OIE 2016)

LSD virus is inactivated by a wide range of disinfectants, including ether (20%), chloroform, formalin (1%) and some detergents e.g. sodium dodecyl sulphate. LSD virus is also susceptible to phenol (2%/15 minutes), sodium hypochlorite (2-3%), iodine compounds (1:33 dilution), Virkon (2%) and quaternary ammonium compounds (0.5%). the detergent SDS, ether, and chloroform (Weiss 1968) (OIE 2016)

The transmission of LSD virus is not well elucidated (Davies 1991). Virus has been isolated from nasal, ocular, and pharyngeal secretions, semen, milk and blood (Thomas and Mare 1945, Weiss 1968). Insect vectors appear to be the main mode of transmission, with the stable fly *Stomoxys calcitrans* likely to be implicated (Hunter and Wallace 2001).

Carcases and meat products: No evidence was found for the persistence of LSDV in the meat of infected animals. AQIS (1999a) has determined that LSDV is unable to be transmitted via meat or meat products. AUSVETPLAN state that meat has never proven a source of infection spread but there remains a theoretical risk of virus survival and spread in frozen meat or packaging. Therefore, meat from infected animals should be disposed of.

Milk and milk products: LSDV may be found in the milk of infected animals (Davies 1991). AQIS (1999b), using data from capripoxviruses in general, has determined that while high temperature / short time pasteurisation is likely to substantially reduce the infectivity of capripoxvirus in milk, it could not be relied on to guarantee total inactivation. There is some evidence that conditions equivalent to the low temperature / long time method inactivate capripoxvirus (62°C for 30 minutes), but the presence of fat, protein and other solids in milk may protect the virus. The low pH of cheese may also be insufficient to inactivate the virus.

Skins, hides and fibres: LSDV has shown infectivity in dried skin lesions on the animal for at least 33 days, and 18 days in scrapings from dry lesions at room temperature (Weiss 1968).

In a draft assessment, AFFA (2001) has concluded that unprocessed skins and hides from susceptible animals in countries with LSD pose a high risk. Given the hardy nature of the virus, it is highly likely to be found on skins from infected animals. Unprocessed or partially processed skins could not be relied on to be free of virus. Only fully processed or fully tanned skins (including 'wet whites' and 'wet blues') are considered to pose no quarantine threat.

Semen/embryos: LSDV is shed for at least 28 days in semen (Annandale et al 2010). Transmission of the virus via semen has been proven under experimental conditions to infect embryos and heifers (Annandale et al 2012). Transmission of the disease via semen has not been shown to occur under field conditions.

Faeces: Reports on the presence or persistence of LSDV in faeces were not found. The virus would be expected to be present as a contaminant.

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Sheep pox / Goat pox

Agent:

Family Poxviridae, genus capripoxvirus

Agent type:

Virus

Persistence and inactivation:

General characteristics: Capripoxviruses are very stable agents in the environment. They are sensitive to sunlight but may persist for up to six months in a cool and dark environment (Geering 1995, Kitching 2000, OIE 2016). Sheep pox virus (SPV) has been shown to persist in vesicular fluid for 2-3 years stored at 0°C or -15°C (Drieux 1975). The virus can survive freeze-thaw cycles but infectivity may be diminished (OIE 2016).

Virus is susceptible to highly alkaline or acid pH (hydrochloric or sulphuric acid at 2% for 15 minutes (OIE 2016).

Capripox viruses are acid labile and loss of infectivity occurs after exposure to pH 3 for 2 hours (Dardiri, 1978). A study using goat virus showed less lability to alkali conditions. There was only a 1 times log₁₀ reduction at pH 8 (Datta and Soman, 1991).

Ferreira (1973) reported the following reductions in infectivity at various time / temperature combinations for SPV suspended in buffer at an initial concentration of 8 log₁₀ TCID₅₀/mL:

- 45°C, two hours, 2.3 log₁₀
- 50°C, 30 minutes, 4 log₁₀
- 50°C, one hour, 6 log₁₀
- 55°C, 30 minutes, 4.6 log₁₀
- 55°C, one hour, virus not detectable
- 60°C, 30 minutes, 5.6 log₁₀
- 60°C, one hour, virus not detectable
- 65°C, five minutes, 5 log₁₀
- 60°C, 30 minutes, virus not detectable.

Other authors have shown similar results, although there are some strain differences (AQIS 1999). The OIE state that virus is inactivated by heat (56°C for 2 hours or 65°C for 30 minutes) (OIE 2016).

SPV is susceptible to a range of disinfectants and detergents including; lipid solvents and acids (e.g. 2% hydrochloric acid, citric acid), 0.1-1.0% hypochlorite, aldehydes, alcohols and iodophors (Dardiri 1978, Animal Health Australia 2011).

SPV is inactivated by phenol (2%) in 15 minutes and is sensitive to: detergents (e.g. sodium dodecyl sulphate); ether (20%); chloroform; formalin (1%); sodium hypochlorite (2-3%); iodine compounds (1:33 dilution); Virkon® (2%); and quaternary ammonium compounds (0.5%).

Sheep pox is transmitted by the respiratory route, infecting animals directly and indirectly via the environment. All excretions and secretions from infected animals may contain virus. The virus is also shed in scabs from lesions in the skin. Mechanical spread by insects is suspected but not confirmed (Geering *et al* 1995, Munz and Dumbell 1994).

Carcases and meat products: Infection with sheep pox is followed by a viraemic phase, so SPV could theoretically be present in muscle, although pH changes associated with rigor mortis would be likely to inactivate the virus.

Kirk (1981) states that sheep pox virus is known to occur in muscle tissue and lymph nodes but the length of its survival there is unknown. He notes that if contamination were to occur during slaughter, the virus could persist for a long time. MacDiarmid and Thompson (1997) observe that such contamination has not been reported and conclude that meat is unlikely to pose a quarantine risk for SPV.

Putrefaction destroys SPV (Blaha 1989). SPV does not infect species other than sheep and sometimes goats, but mechanical transmission via insects may occur (Munz and Dumbell 1994). These facts add weight to the argument that the meat and internal organs of infected sheep carcasses are unlikely to pose an infective risk. The skins and wool, however, do pose a problem.

AUSVETPLAN (Animal Health Australia 2011) stipulates either burning or burial of infected carcasses. Spraying with phenol, and strict control of vermin, may be required if a delay is likely between slaughter and disposal.

Milk and milk products: The virus may be isolated in milk in the early stages of the disease when the fever is evident (Davies 1991a), although infected milk plays a minor role in transmission of the disease under natural conditions (Munz and Dumbell 1994).

Thermal inactivation data (see above) suggest that while high temperature / short time pasteurisation is likely to reduce that capripoxvirus in milk significantly, it is possible that milk or milk products could contain infective virus after treatment. Milk is more protective than the buffer solutions in which the data were derived. The low pH of cheese could not be relied upon for inactivation (AQIS 1999).

AUSVETPLAN (Animal Health Australia 2011) require milk from suspect animals to be destroyed using heat, acid or by being buried.

Skins, hides and fibres: SPV can survive in wool or hair of recovered animals for up to three months (Geering *et al* 1995). In a draft assessment, AFFA (2001) has concluded that unprocessed skins and hides from susceptible animals in countries with SPV pose a high risk. Given the hardy nature of the virus, it is highly likely to be found on the skin of infected animals. Unprocessed or partially processed skins could not be relied on to be free of virus. Only fully processed or fully tanned skins (including 'wet whites' and 'wet blues') are considered to pose no quarantine threat.

AUSVETPLAN (Animal Health Australia 2011) require any skins, wool or fibre which may have been contaminated to be burned or buried.

Semen/embryos: No references were found on the presence or persistence of SPV in semen or on embryos. The closely-related lumpy skin disease virus has been found in semen up to 22 days after infection (Davies 1991b). Orchitis has been reported in goats (Merza and Mushi 1990), and intrauterine transmission can occur during cowpox (Mayr and Czerny 1990). AQIS (2000) concludes that there is a risk of contamination of semen or embryos. Semen and embryos should not be collected from animals that are subject to disease control restrictions (Animal Health Australia 2011).

Faeces: No reports were found on the presence or persistence of LSDV in faeces. The virus would be expected to be present as a contaminant. Manure is normally incinerated (Munz and Dumbell 1994).

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Reoviridae

African horse sickness

Agent:

Family Reoviridae, genus orbivirus

Agent type:

Virus

Persistence and inactivation:

General characteristics: The characteristics of African horse sickness virus have been well summarised by Coetzer and Erasmus (1994). AHSV has been reported as persisting for:

- Ten minutes at 55-75°C
- Three months in a medium containing calf serum at 4°C (although infectivity is rapidly lost at -25°C unless a stabiliser is added)
- At least six months at 4°C in saline containing 10% serum
- More than two years in putrid blood
- Twelve months in washed infected erythrocytes stored at 4°C.

African horse sickness is spread by *Culicoides* midges. It is not contagious. Mechanical transmission by biting flies may play a very minor role but the virus is sensitive to high temperatures and desiccation (Coetzer and Erasmus 1994).

The optimal pH for survival for AHSV is 7.0-8.5, with greatest lability at the acid end of this range.

Virus is resistant to lipid solvents such as ether. AHSV is inactivated by formalin (0.1%) for 48 hours, beta-propiolactone (0.4%) and binary ethyleneimine. Virus is inactivated by acetic acid (2%), potassium peroxydisulfate/sodium chloride-VirkonS® (1%) and sodium hypochlorite (3%). (OIE 2016).

Chlorine and iodine and quaternary ammonium compounds are effective against AHSV. The effective concentration of quaternary ammonium compound was the lower than for the iodine and quaternary ammonium disinfectants examined (Junsuke et al 2000).

Carcases and meat products: No references were found on the persistence of AHSV in carcasses or meat products. Mellor (pers comm) believes that the work had not been done. The relative acid lability of the virus suggests that it would be inactivated by the pH changes accompanying rigor mortis.

Dogs have become infected by feeding on the carcasses of infected animals (Van Rensburg 1981).

Skins, hides and fibres: The means of transmission of AHS suggests that the risk of skin, hides or fibres being infective is almost zero. In a draft assessment, AFFA (2001) has concluded that skins and hides pose a negligible quarantine risk for AHS.

Semen/embryos: Virus can be found in semen, urine and nearly all secretions during viraemia, but no studies have documented transmission (OIE, 2016).

Faeces: No references were found on the presence of AHSV in faeces.

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Bluetongue

Agent:

Family Reoviridae, genus orbivirus

Agent type:

Virus

Persistence and inactivation:General characteristics:

Bluetongue virus is very stable in blood and tissue specimens. Viral stability is enhanced in the presence of protein (viable virus has survived years in blood stored at 20°C) (OIE 2016). In the absence of extraneous protein, it is extremely unstable at high temperatures and is readily inactivated by heat (50°C in 3 hours and 60°C in 15 minutes) (OIE 2016). BTV is unstable below pH 6.5 and above pH 8.0 (OIE 2016). Virus is readily inactivated by disinfectants containing acid, alkali, sodium hypochlorite and iodophors. It is relatively resistant to UV and gamma radiation, and lipid solvents such as ether and chloroform (Verwoerd and Erasmus 1994).

Geering *et al* (1995) state that bluetongue is transmitted via *Culicoides* midges, and that there is no transmission by direct contact between animals in the absence of the vector, nor by indirect means. There is very little excretion or secretion of virus by infected animals. Transmission by oral or aerosol means is highly unlikely to occur and products even from infected animals (except for semen) “can be disregarded as a source of infection” (Verwoerd and Erasmus 1994).

Carcases and meat products: No reports were uncovered regarding the persistence of bluetongue in carcasses or meat products. The acid lability of BTV would suggest inactivation of the virus during post-mortem maturation of the carcass. AQIS (1999a) has determined that bluetongue “is unable to be transmitted by meat or meat products”. Animal Health Australia (2015) state in AUSVETPLAN that bluetongue does not survive outside living vectors or hosts.

Milk and milk products: No reports were uncovered of bluetongue virus being shed in milk. AQIS (1999b) has determined that milk does not pose a quarantine threat for BT and this is supported in AUSVETPLAN (Animal Health Australia 2015).

Skins, hides and fibres: The means of transmission of BT suggests that the risk of skin, hides or fibres being infective is almost zero. In a draft assessment, AFFA (2001) has concluded that skins and hides pose a negligible quarantine risk for BT.

Semen/embryos: The risk of transmission of bluetongue via semen or embryos has been reviewed by several authors (Roberts *et al* 1993, Sellers 1983).

BTV has been found in bull semen, during the period of viraemia, and is infective either by natural or artificial insemination (Verwoerd and Erasmus 1994, Roberts *et al* 1993). The period of viraemia varies to a generally accepted maximum of 50 days in cattle and 20 days in sheep (Geering *et al* 1995). The number of organisms found in the semen of cattle is sufficient to infect heifers, at least during a 25-day period from about day 10 of infection (Sellers 1983).

Roberts *et al* (1993) cite a series of papers by Luedke *et al* (Luedke *et al* 1977, 1983, Luedke and Walton, 1981) describing persistent viraemia and shedding of BTV in semen by a bull

infected in utero. The bull was negative on serological testing and viraemic to 11 years of age. Bovine embryos with an intact zona pellucida are protected from BTV infection however, without a zona pellucida, they are readily infected and will degenerate (Bowen *et al* 1982). The likelihood of intact embryos carrying intracellular infection is therefore very low, although BTV could be present in the embryonic environment in association with cellular blood elements. Washing embryos ten times according the International Embryo Transfer Society manual (Stringfellow *et al.* 1990) provides 95% confidence that the percentage of BTV-contaminated embryos from viraemic donors would be no larger than 1% (Sutmoller *et al.* 1997).

The situation with sheep is less clear. Hare *et al* (1988) found BTV in the semen of rams but were unable to transmit infection to ewes bred to these rams. These authors also reported non-transmission of infection by embryos washed ten times. Gilbert *et al* (1987) found that BTV adhered to the zona pellucida of ovine embryos, and successfully transmitted infection via embryos from viraemic ewes. However, the embryos were only washed three to four times. In another study, ovine embryos exposed to BTV *in vitro* were not disinfected by ten washes, but the viability of the embryos was not clear (Singh *et al* 1997).

Faeces: No reports of BTV appearing in faeces were uncovered during this review.

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Equine encephalosis

Agent:

Family Reoviridae, genus orbivirus

Agent type:

Virus

Persistence and inactivation:

Little information could be found on the persistence and inactivation of this virus. Erasmus *et al* (1970) could reduce infectivity *in vitro* with 0.5% trypsin, or with exposure to pH 3.0 for one hour at 37°C. The virus was totally inactivated after 5 minutes at 60°C, with “considerable loss” of infectivity at 56°C after one hour. It was resistant to chloroform.

Equine encephalosis virus is spread by arthropods, with *Culicoides* spp midges appearing to be an important vector (Coetzer and Erasmus 1994).

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Retroviridae

Jembrana disease

Agent:

Family Retroviridae, genus lentivirus

Agent type:

Virus

Persistence and inactivation:

Very little research of direct interest has been conducted. In summary, Jembrana disease virus appears to be quite unstable, a characteristic consistent with other lentiviruses such as human immunodeficiency virus (Wilcox, pers comm). The virus is sensitive to diethyl ether (Kertayadnya *et al* 1996). In the absence of direct research, information on pulmonary adenomatosis and maedi-visna viruses is likely to provide the best indicator of the properties of Jembrana disease virus.

Jembrana disease is thought to be transmitted mechanically by biting insects and/or during mass vaccination programs (Geering *et al* 1995).

Carcases and meat products: There is no published information on this aspect of the disease. AQIS (1999a) has concluded that Jembrana disease “does not appear likely to be transmitted in meat or meat products”. The virus survives for several months in infected spleens kept at -22°C in Indonesia, although there appears to be substantial loss of infectivity (Wilcox pers comm). A titration study reported by Kertayadnya *et al* (1996) showed a decline in virus particles from 10^8 to 10^2 ID₅₀/mL in plasma stored at 4°C for 24 hours. Rapid freezing / thawing of plasma at -70°C caused a reduction in titre from 10^8 to between 10^3 and 10^4 ID₅₀/mL, a level which was maintained during storage at -70°C for 2 months.

Milk and milk products: JDV has been found in the milk of infected cows during the viraemic phase, and milk containing JDV has been shown to be capable of initiating disease (Soeharsono *et al* 1995). There is limited information on how long the virus is excreted in milk. Research on other lentiviruses suggests that normal pasteurisation procedures would inactivate the virus. AQIS (1999b) has concluded that, in the absence of further information, dairy products must be assumed to present a quarantine risk for JDV, and will only allow milk and milk products from countries with Jembrana disease provided that the milk is pasteurised.

Skins, hides and fibres: JDV is unlikely to be stable on skins or fibres for a prolonged period. In a draft assessment, AFFA (2001) has concluded that “the likelihood of transmission of Jembrana disease via hides or skins would be negligible”.

Semen/embryos: JDV was not detected in the semen of experimentally infected bulls before or immediately after the febrile phase by Soeharsono *et al* (1995). The risk of transmission by semen appears to be low, but there is insufficient information to entirely rule it out.

Faeces: Excretion of virus in faeces is not mentioned in the literature as a feature of the disease.

References:

AFFA (Department of Agriculture Fisheries and Forestry – Australia) 2001, *Import risk analysis. Skins and hides. Draft report, August 2001*, AFFA, Canberra.

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Maedi-visna

Agent:

Family Retroviridae, genus lentivirus

Agent type:

Virus

Persistence and inactivation:

Maedi-visna virus is readily inactivated by UV radiation, ethyl ether, chloroform, formaldehyde, ethanol, phenol and trypsin (Petursson *et al* 1990, DeMartini pers comm). Virus infectivity is relatively stable between pH 5.1 and 10. The following reductions in infectivity of virus suspended in buffer at 19-21°C have been reported by Thormar (1965):

pH	Reduction in infectivity	Time
9.4	1 log ₁₀	4 days
7.7	1 log ₁₀	7 days
5.1	1 log ₁₀	1 day
4.2	1 log ₁₀	1.5 hours (maedi), 1 hour (visna)
3.2	4 log ₁₀	0.5 hours (maedi)
3.2	5.5 log ₁₀	0.5 hours (visna)

MVV is stable for months at -50°C and relatively resistant to repeated freezing and thawing (Petursson *et al* 1990). One log₁₀ (90%) reduction in infectivity was reported at 50°C in 1% serum (pH 7.3-7.5) after 10 minutes, and a 5 log₁₀ reduction at 56°C after 10 minutes (Thormar 1965).

MV is transmitted by direct contact, presumably via the respiratory route, but also through milk. Indirect transmission through water contaminated with faeces may occur but is considered unimportant (Geering *et al* 1995).

Carcases and meat products: This review did not uncover any specific studies on the persistence of MVV in carcasses or meat products. The lack of primary data was confirmed by DeMartini (pers comm). AQIS (1999a) and MacDiarmid and Thompson (1997) have concluded that MVV does not present a quarantine risk for transmission by meat or meat products.

Milk and milk products: The principal means of transmission of maedi-visna is via milk and colostrum (Pepin *et al* 1998). AQIS (1999b) noted that the heat inactivation data of Thormar (1965) should be interpreted with caution regarding milk, because the virus is present in monocyte/macrophage cells. Pasteurisation of goat milk at 56°C has been effective in the inactivation of the closely related caprine arthritis-encephalitis virus. Pepin *et al* (1998) recognised heating of ewe colostrum at 56°C for one hour, and pasteurisation of milk, as components of a MV eradication program within a flock. AQIS (1999b) found no direct data on the effect of HTST pasteurisation on milk containing MVV, nor could this review find any.

The data of Thormar (1965) suggested that the pH range of cheeses would inactivate the virus. AQIS (1999b) has concluded that, in the absence of further information, dairy products must be assumed to present a quarantine risk for JDV, and will only allow milk and milk products from countries with maedi-visna provided that the milk is pasteurised.

Skins, hides and fibres: There does not appear to be any primary data on the presence of MVV on sheep skins or wool. MVV is present in all body fluids, so contamination could not be ruled out, and there is horizontal transmission of MVV via respiratory secretions (Pepin *et al* 1998). However, the relative fragility of MVV suggests that the risk would be very low. In a draft assessment, AFFA (2001) concluded that “the mode of transmission of maedi-visna is such that hides and skin are unlikely to be a significant risk factor in such transmission”.

Semen/embryos: MVV has been detected in the semen of rams simultaneously infected with MVV and ovine brucellosis (de la Concha-Bermejillo *et al* 1996), possibly because of leucocytospermia. MVV was not found in uterine washes or washed embryos from infected ewes (Woodall *et al* 1993). No studies have definitively demonstrated transmission of MVV by venereal means (Pepin *et al* 1998). AQIS (2000) concluded that the risk of introducing MVV to Australia via embryos was small, and via semen moderate.

Faeces: There has been a report of transmission of maedi-visna via drinking water contaminated with faeces, but the oral/faecal route is not considered to be important part of the epidemiology (Geering *et al* 1995).

References:

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Pulmonary adenomatosis

Agent:

Family Retroviridae

Agent type:

Virus

Persistence and inactivation:

Pulmonary adenomatosis virus has not been cultured *in vitro*, so there is very little primary information on the survival and inactivation of the virus. It does not survive exposure and desiccation for long (Verwoerd 1990).

Cousens *et al* (2009) found lung fluid from infected sheep contained virus and that transmission could be achieved following inhalation of this fluid. The amount of fluid produced can vary from 0-500mls/day for a week. Examinations under conditions that mimic natural conditions suggest intact virions may persist for several weeks in the environment.

When dried, the virus was detectable for 3 days but little remained by 14 days at any temperature. In wet conditions at 20°C, no loss of virus occurred in 3 days and 12% remained after 14 days. In wet conditions at 37°C, 13% of virus particles survived to 3 days and no detectable live virus was found at 14 days.

The disease is thought to be transmitted by the respiratory route in aerosols (Geering *et al* 1995).

Carcasses and meat products: No primary information is available on the survival of PAV in carcasses or meat products. Verwoerd (1990) states that the infection appears to remain localised in the lung, and that “no evidence has been found to date of virus or viral antigens in any other organ, including the blood.” Since then, Holland *et al* (1999) have identified proviral RNA and DNA in peripheral blood mononuclear and other cells, so the absence of virus in the muscle could not be dismissed. However, the virus is unlikely to survive long in the carcass given its relative fragility. AQIS (1999a) has concluded that PAV does not present a quarantine risk for transmission by meat or meat products.

Milk and milk products: Pulmonary adenomatosis virus is only known to be spread by respiratory droplets (Verwoerd 1990). It is not considered to pose a quarantine hazard in dairy products (AQIS 1999b).

Skins, hides and fibres: There does not appear to be any reported data on the presence of pulmonary adenomatosis on sheep skins or wool. Presence of the virus on skin is unlikely, except where contamination from infected aerosols has occurred. In a draft assessment, AFFA (2001) concluded that “the mode of transmission of pulmonary adenomatosis is such that hides and skin are unlikely to be a significant risk factor in such transmission”.

Semen/embryos: Parker *et al* (1998) demonstrated infection-free transfer of embryos from dams in infected flocks, or from uninfected dams mated to an infected sire. This appears to be the only study on venereal transmission of PAV. AQIS (2000), however, noted that “any concurrent disease process in a pulmonary adenomatosis infected ram which increases the mononuclear content of semen may lead to the production of pulmonary adenomatosis infected semen”. It concluded that the risk of introducing PAV to Australia via embryos was small, and via semen moderate.

Faeces: No reports were found to indicate the presence of PAV in faeces.

References:

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Rhabdoviridae

Australian lyssavirus

Agent:

Family Rhabdovirus, genus lyssavirus

Agent type:

Virus

Persistence and inactivation:

No studies on the survival properties of Australian lyssavirus were uncovered during this review (the absence of any information was confirmed by McColl, pers comm and Field, pers comm). No further references were found in the 2017 review.

Little is known about persistence of lyssavirus. For classical rabies, the key relevant persistence features are as follows:

- The virus is comparatively fragile and does not survive long periods outside the host.
- The virus is stable for several months at 0-4 °C but is rapidly inactivated by heat, direct sunlight and lipid solvents.
- The virus is stable at pH 5-10. The virus survives in saliva up to 24 hours in temperate climates.

Lyssaviruses have not been found in faeces. Australian bat lyssavirus RNA has been detected in a sample of bat urine but the risk of infection via urine is unclear. Dead bats should be disposed of via incineration or burial if incineration is not possible.

Bat lyssavirus is inactivated by most organic solvents, oxidising agents and surface active agents (quaternary ammonium compounds, soaps and detergents). Oxidising agents such as hypochlorite and virkon may be used on inanimate objects. Quaternary ammonium compounds are also useful for personal disinfection. Bites from infected bats should be washed for 5 minutes with warm soapy water but not scrubbed. After washing, disinfectant (either alcoholic or halide compound) should be rapidly applied.

Fomites are not infectious. Virus is highly labile therefore environmental contamination by infected animals is negligible. No environmental disinfection is required. Fresh saliva should be cleaned with disinfectant or warm soapy water.

Fresh fruit harvested from trees inhabited by bats should be washed with soapy water before eating (Animal Health Australia 2008).

References:

A review of bat lyssaviruses can be found in: McColl, K.A., Tordo, N., & Setien, A. 2000, 'Bat lyssavirus infections', *Rev sci tech Off int Epiz*, vol. 19(1), pp.177-196.

Animal Health Australia 2008. 'AUSVETPLAN Operational Procedures Manual: Decontamination (Version 3.2),' In: *Australian Veterinary Emergency Plan (AUSVETPLAN), Edition 3*, Primary Industries Ministerial Council, Canberra, ACT

Rabies

Agent:

Family Rhabdovirus, genus lyssavirus

Agent type:

Virus

Persistence and inactivation:

General characteristics: Rabies virus (RV) is a fragile virus, surviving only for short periods outside the host. It is inactivated by heating to 56°C for 30 minutes, but can be preserved for years at temperatures less than -60°C or by freeze drying and storing at -4°C (Swanepoel 1994). It is stable at pH 5-10 at 4°C, and labile at pH 3 in 30 minutes.

The virus is sensitive to pH extremes (<3 and >11) (OIE 2016). Rabies virus is susceptible to UV light and to lipid solvents (soapy water, ether, chloroform, acetone), ethanol 45-75%, quaternary ammonium compounds (e.g. 0.2% cetrимide), and iodine preparations 5-7% (AFFA 2001a, Bowen-Davies & Lowings 2000). However, the rate of inactivation of RV by physical and chemical conditions is greatly modified by the stabilising effects of polypeptides and other compounds (Wandeler pers comm, Michalski *et al* 1976).

Carcases and meat products: Transmission of rabies between animals through the ingestion of contaminated tissues has been documented (see reviews of non-bite transmission of rabies by Afshar 1979 and Swanepoel 1994). However, no descriptions of the survival of RV in carcasses or meat products were found during this review, and MacDiarmid and Thompson (1997) state that rabies virus has never been isolated from meat. But AUSVETPLAN (Animal Health Australia 2011) requires all products and by-products of infected animals to not enter the food chain. The virus does not survive for more than 24 hours in dead animals when temperatures reach 21°C but it is highly resistant over extended periods at low or freezing temperatures (OIE 2016).

Wandeler (pers comm) advised that the fragility of rabies viruses has “probably precluded detailed studies of their inactivation in carcasses”. There is an assumption that virus on the exposed surfaces of the carcass would be inactivated within a few hours, whilst infectivity of internal organs is lost within a few days in summer or many weeks in winter. Infectivity persists longest in organs with a high ante-mortem load of virus (CNS, salivary glands, pancreas, and adrenals). Persistence would be expected to be short-lived under most Australian conditions given the heat lability of the virus (see AFFA 2001a). However, AUSVETPLAN regard the survival of virus in saliva of dead animals for a period as possible; especially in temperate climates (Animal Health Australia 2011).

Fooks (pers comm) has confirmed the paucity of data on persistence of rabies in carcasses and animal products, and speculated that the virus “would not survive for long (a few weeks)”. He understood that rendering any carcass would completely inactivate the virus.

MacDiarmid and Thompson (1997) conclude that the probability of introducing rabies in meat or meat products “must be considered remote”.

Milk and milk products: Transmission of rabies via suckling has been reported in several species (Afshar 1979, Swanepoel 1994). These reports have been described as “rare and anecdotal” and milk products are not considered to pose an import risk to Australia for rabies (AQIS 1999a).

Skins, hides and fibres: This review did not uncover any specific reports on the persistence of RV on skins, hides or fibres. The risk of RV being present on product for any period is likely to be very small, given the susceptibility of the virus to drying and UV light. In a draft report, AFFA (2001b) has determined that “the mode of transmission of the disease is such that hides and skins are unlikely to be a significant risk factor in the transmission of rabies”.

Semen/embryos: There are no reports of rabies virus having been isolated from or transmitted by semen in livestock. AQIS (1999b, 2000) have concluded that the risk of this occurring in cattle or pigs is very low, although it notes that rabies virus has been isolated from the testis of a vampire bat and that bovine serum can stabilise the infectivity of the virus during freezing and thawing. Similarly, rabies virus has been demonstrated in one embryo, the uterus and ovaries of a skunk, so contamination of bovine embryos cannot be entirely ruled out. AQIS requires only that semen and embryos to be imported must come from donor animals showing no signs of clinical signs of rabies during and for 15 days after collection.

Faeces: No reports were found of the presence of rabies virus in faeces.

Necropsy equipment: Aiello et al (2015) studied selected disinfectants on rabies-contaminated equipment also containing organic matter. None of the agents tested under label conditions were effective. Biguanide and quaternary ammonium compounds were ineffective under all conditions. Oxidising or phenolic compounds used off label were effective. The critical importance of the effective removal of organic material before chemical disinfection of equipment and surfaces is emphasised by these results.

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Vesicular stomatitis

Agent:

Family Rhabdovirus, genus vesiculovirus

Agent type:

Virus

Persistence and inactivation:

General characteristics: As with other Rhabdoviridae, vesicular stomatitis virus (VSV) is a relatively fragile agent. Fong and Madin (1954) reported stability of VSV between pH 4-11.6, although Wooley and Gilbert (unpublished, cited in Wooley *et al* 1981) inactivated VSV in 2 hours at pH 4-5. It is inactivated by temperatures over 50°C but can survive in soil at 4-6°C (Hanson and McMillan 1990). VSV may be preserved for years at -60°C and by freeze-drying under vacuum (Hanson 1981).

Virus is inactivated by heat (58°C for 30 minutes) but can survive for long periods at low temperatures. Virus is stable between pH 4.0-10.0 (OIE 2016). Virus is readily inactivated by sunlight.

The virus is sensitive to formaldehyde, ether and other organic solvents; chlorine dioxide, 1% formalin, 1% sodium hypochlorite, 70% ethanol, 2% gluteraldehyde, 2% sodium carbonate, 4% sodium hydroxide, and 2% iodophore disinfectants (OIE 2016).

Zimmer B *et al.* (2013) showed that VSV in suspension was stable over several weeks at 40°C. When dried on surfaces (e.g. stainless steel, glass), VSV survived at least 6 days at ambient temperatures. Virus was rapidly inactivated at temperatures of 55 °C. Virus is highly sensitive to common disinfectants.

The epidemiology of VS is poorly understood. VSV is known to be transmitted directly via the transcutaneous or transmucosal route (OIE 2016). Arthropods are probably important in transmission, but viraemia is not a feature of the disease in pigs or horses. The virus is thought to enter the animal only via insect bites or abrasions (Wilks 1994, Letchworth *et al* 1999). Low-grade viraemia has been observed only in experimentally-infected cattle (Orrego *et al* 1987).

Carcases and meat products: VSV is not found in any edible tissues (Hanson 1981). No reports were found on the presence of the virus in meat or meat products (see also AFFA 2001a).

Wooley *et al* (1981) reported the inactivation of VSV within two hours of being inoculated into a can of food waste fermenting with *Lactobacillus acidophilus*. The virus could not be recovered even at 5°C, suggesting the low pH (4-5) was contributing to inactivation.

Milk and milk products: No reports were found of VSV in milk. Hanson (1981) states that “the virus, when present in milk, does not survive pasteurisation”. DPIE (1996) has interpreted this to mean that VSV is present in raw milk, but AQIS (1999a) stated that an extensive review of the literature had failed to reveal any original account of VSV being excreted or transmitted in milk and concluded that milk did not present a quarantine hazard for the introduction of VSV.

However, vesicles of VS can be present on the teat of infected cows (Hanson 1981). These could be expected to discharge virus into the milk.

Cliver (1973) found that VSV, artificially added at 10^5 plaque-forming units per 2 kg of pre-starter samples, was not recoverable in curd before pressing during a stirred-curd cheddar procedure.

Skins, hides and fibres: In a draft assessment, AFFA (2001) has concluded that, because of the method of transmission and the unstable nature of the virus, spread of VSV via skins is unlikely to occur. No specific quarantine measures are therefore considered necessary.

Semen/embryos: This review did not uncover any reports of VSV having been isolated from or transmitted by semen. AQIS (1999b, 2000) have concluded that the risk of transmission of VSV by artificial insemination is very low, although equipment such as straws and containers could be readily contaminated. Extrinsic contamination of uterine flushes by the virus may occur. VSV adheres to the zona pellucida of bovine embryos and neither washing nor treatment with trypsin can be relied upon for disinfection (Lauerman *et al* 1986, Stringfellow *et al* 1989).

Sutmoller and Wrathall (1997) noted that the brief viraemia observed in some cases of experimentally-infected cattle may lend some suspicion to the contamination of embryos, but concluded that the risk was close to zero.

Faeces: VSV does not appear in faeces or urine (Letchworth *et al* 1999).

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Togaviridae

Getah virus disease

Agent:

Family Togaviridae, genus alphavirus, Semliki Forest antigenic complex

Agent type:

Virus

Persistence and inactivation:

Getah virus is spread by mosquitoes (Geering *et al* 1995). Disease can be induced experimentally in horses by subcutaneous, intramuscular and intranasal inoculation. Infection has been induced in pigs by intramuscular inoculation, but while spread by direct contact is conceivable, it appears to be unimportant in the field (Kono 1988). In mice, there is horizontal spread between littermates and vertical offspring via milk (Kono 1988).

Infected horses have a short viraemia, with virus localising in a wide range of tissues including lymph nodes, lung, spleen, liver and bone marrow (Kamada *et al* 1981). Sentsui and Kono (1980) did not find infective Getah virus in faeces or urine of infected horses.

No references were found on the persistence of Getah virus in meat or other animal products. The closely related Western, Eastern and Venezuelan equine encephalomyelitis viruses are reported to be extremely fragile, disappearing from infected tissues within a few hours after death (Radostits *et al* 2000). No new references were found in the 2017 review.

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Porcine reproductive and respiratory syndrome

Agent:

Family Togaviridae, genus arterivirus

Agent type:

Virus

Persistence and inactivation:

General characteristics: The survival properties of porcine reproductive and respiratory syndrome virus have been summarised by AFFA (2001a).

PRRSV appears to be most stable in the pH range 5.5-6.5 (Bloemraad *et al* 1994), with Benfield *et al* (1992) reporting 90% inactivation of the virus outside the pH range 5-7.

Heat stability studies on the European strain of the virus have shown survival for at least 72 hours at 4°C or -20°C, but 93% loss of infectivity after 72 hours at 25°C (Alstine *et al* 1993). The US strain is stable for at least 18 months at -70°C, for at least one month at 4°C, and loses 50% of viability after 12 hours. Complete inactivation was achieved after 48 hours at 37°C and after 45 minutes at 56°C (Benfield *et al* 1992).

Virus can be recovered from muscle 0-24 hours after slaughter but not from muscle held at 4°C for 48 hours. The virus survives freezing in cell culture for prolonged periods. Virus can survive for one month in muscle at -20°C. AUSVETPLAN (Animal Health Australia 2006) state that virus can survive for several weeks in bone marrow at 4°C.

Bloemraad *et al* (1994) found in culture medium at pH 7.5 the virus half-life was:

- 140 hours at 4°C
- 20 hours at 21°C
- 3 hours at 37°C
- 6 minutes at 56°C

This is supported by the work of Jacobs *et al.* (2010) who found similar virus half-life:

- 155.5 hours at 4°C
- 84.8 hours at 10°C
- 27.4 hours at 20°C
- 1.6 hours at 30°C

A review of the virus (Cho & Dee 2006) concluded that peroxygen, quaternary ammonium chlorides and quaternary ammonium-glutaraldehydes combinations are highly effective in disinfecting surfaces. AUSVETPLAN list the virus as being rapidly inactivated by solvents such as chloroform and ether. The virus does not persist on equipment more than 24 hours due to drying (Animal Health Australia 2006).

The virus persists in the environment for up to three weeks (Geering *et al* 1995). Transmission of PRRS occurs via aerosols. Windborne spread is a feature of the disease particularly in conditions of high humidity, low wind speed and low ambient temperature (Animal Health Australia 2006). Virus has been isolated from faeces, urine, and semen (Done *et al* 1996). Rodents do not appear to be a reservoir of the disease (Hooper *et al* 1994).

Magar (pers comm) advised no direct work had been conducted on disposal methods for infected carcasses or animal products, and none were uncovered during this review.

Carcasses and meat products: After infection, PRRS probably spreads during viraemia to various organs and tissues (Magar *et al* 1995).

Magar *et al* (1995) looked for PRRSV in the tissues of pigs who were either experimentally infected or who came from infected herds. In the first group, virus was detected at 7 days post-inoculation lungs, tonsils, lymph nodes and muscle tissue. Virus was not detected in muscle at 14 days but was found in lungs and tonsils.

No virus could be found in the second group, seropositive pigs from known infected herds, in pools of tissue from 44 pigs. (Virus was found only in one lymph node pool, and it could not be grown in alveolar macrophages.) The time since the sampled pigs had been exposed to the virus was not known, but was probably weeks to months. The authors concluded that it was unlikely that the disease would be transmitted by pork.

European and American strains of PRRSV have been demonstrated in pooled samples of ham muscle and bone marrow in pigs slaughtered six days post-infection (Frey *et al* 1995). The pooled muscle bone marrow samples retained infectivity for several weeks when at 4 °C and at least one month when stored at -20 °C.

A study by Larochelle and Magar (1997), using a sensitive PCR assay, failed to detect PRRSV in 438 muscle tissue homogenates from 73 different lots of packaged meat collected over seven months.

In a study commissioned by AQIS at Lelystad ID-DLO (AFFA 2001a), European and American strains of PRRSV were successfully transmitted to receiver pigs by feeding muscle tissue from experimentally-infected pigs. Infection was confirmed by virus isolation and antibody detection three weeks after feeding.

A review (Pharo and Cobb 2011) concluded that there was sufficient data to construct a model that illustrated that trade in commercially produced pig meat was unlikely to result in spread of PRRS. Cooked pig meat (with or without bone) using a cooking process that involved a minimum core temperature of 4°C for 11 minutes and dry curing of pig meat for a period beyond 140 days is regarded to provide product of very low risk for transmission of virus (AFFA 2004)

Skins, hides and fibres: No specific references were found on this aspect of the disease. In a draft assessment, AFFA (2001b) determined that these products do not pose a quarantine risk for PRRS.

Semen/embryos: PRRSV has been detected in semen from three days, and for up to 92 days after infection in some pigs (Christopher-Hennings 1995).

Experimental transmission of PRRSV to gilts in semen was recently achieved by Gradil *et al* (1996). Seroconversion was demonstrated in gilts inseminated with semen from boars that had been inoculated with PRRSV.

Faeces: PRRSV has been isolated from faeces of infected pigs (Done *et al* 1996).

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Agent:

Family Togaviridae, genus alphavirus

Agent type:

Virus

Persistence and inactivation:

Equine encephalomyelitis viruses are transmitted by mosquitoes (Radostits *et al* 2000).

The level of viraemia produced in horses is very high (Radostits *et al* 2000), but has not been reported to exceed 5 days in duration. The virus can be isolated from brain, although this has proven unrewarding in some cases, or serum, which should be taken from pre-clinical febrile horses (Walton 1992). The virus is present in saliva and nasal discharge.

The thermal deactivation point for alphaviruses is 58 °C and virus half-life is 7 hours at 37°C. The virus is stable in pH 7-8 but quickly inactivated at acidic pH. (OIE 2016).

(Fitzgibbon *et al.* 2008) found that Venezuelan equine encephalitis (VEE) virus was stable in distilled, deionized water for 21 days and concluded that the virus could remain viable for days after release into water or snow.

(Guzman *et al.* 2005) found that VEE could be detected for 40 days on air dried filter paper discs stored at room temperature. The viruses are inactivated by common disinfectants sensitive to organic solvents and detergents, 1% sodium hypochlorite, 70% ethanol, 2% glutaraldehyde and formaldehyde.

These viruses are sensitive to sunlight and heat (moist or dry heat). The virus survives best in cool, moist dark conditions (OIE 2016).

Sagripani (2010) found that relatively little overnight inactivation should be expected when virus is deposited on glass. The inactivation kinetics showed dried VEE virus took 11.4 days to reduce virus load to 10% and 42.7 hours to reduce virus load to 37%.

Monath and Trent (1981) state that serum or tissue suspensions should be preserved at -70°C. Alphaviruses will also survive at ambient temperatures for several days in blood dried on paper discs. Equine encephalomyelitis viruses are extremely fragile and disappear from infected tissues within a few hours after death (Radostits *et al* 2000).

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Other viruses

African swine fever

Agent:

Unclassified DNA virus like iridoviruses and poxviruses

Agent type:

Virus

Persistence and inactivation:

General characteristics: African swine fever virus is a very hardy agent. Stability has been demonstrated in various strains for:

- six years at 5°C with no light
- eighteen months in serum at room temperature
- up to a month at 37°C
- 3.5 hours at 56°C (although serum can normally be sterilised after 30 minutes at 60°)

Virus is highly resistant to low temperatures. It is heat inactivated by 56°C in 70 mins or at 60°C in 20 mins. It is inactivated by pH <3.9 or >11.5 in serum-free medium (serum increases the resistance) e.g. at pH 13.4 resistance lasts up to 21 hrs without serum and 7 days with serum.

ASFV is most stable at pH 4-10, but residual infectivity has been demonstrated at pH 3.1 after 22 hours, at pH 3.9 in serum after three days, and at pH 13.4 in serum after a week.

The virus is resistant to proteases (trypsin, pepsin), to nucleases and to putrefaction. It is very sensitive to lipid solvents and detergents, as well as oxidising agents such as hypochlorite and substituted phenols. Beta-propiolactone, acetyl-ethyleneimine and glycidaldehyde destroy infectivity within one hour at 37°C, and formalin (0.5%) within about four days (Bengis 1997, Farez and Morley 1997, Plowright and Parker 1967, Plowright *et al* 1994). Virus is susceptible to ether and chloroform and inactivated by 0.8% NaOH (30 minutes), hypochlorites – 2.3% chlorine (30 minutes), 0.3% formalin (30 minutes), 3% ortho-phenylphenol (30 minutes) and iodine compounds (OIE, 2016).

Krug *et al* (2011) demonstrated that sodium hypochlorite (1000 ppm) and citric acid (1%) resulted in complete disinfection of surface-dried (on plastic and metal) high titer viral samples.

Krug *et al* (2012) demonstrated that sodium hypochlorite (2000ppm) and 2% citric acid for 2 minutes inactivated ASFV on wood/porous surfaces.

Shirai *et al* (2000) states that chlorine was effective against ASFV and three other enveloped viruses at concentrations of 0.03% to 0.0075%, and a dose response was observed. Iodine was very effective at concentrations of 0.015% to 0.0075%, but a dose response was not observed. Quaternary ammonium compound was very effective in low concentration of 0.003% against ASFV and the other three tested enveloped viruses.

ASFV is present in nasal, oral, pharyngeal, conjunctival, genital, urinary and faecal secretions and excretions. Transmission occurs by direct contact and via the environment (Plowright *et al* 1994). Virus remains viable for long periods in blood, faeces and tissues; especially infected, uncooked or undercooked pork products and can multiply in vectors (OIE, 2016).

Carcases and meat products: Kowalenko *et al.* (1965) reported that ASFV persisted for 150 days at 4°C and for 104 days at -4°C in skeletal muscle, and for six months in bone marrow at -4°C. Persistence of ASFV in meat products has been comprehensively reviewed by Farez and Morley (1997). The virus has been shown to survive for up to:

- 140 days in Iberian and white Serrano hams (Mebus *et al* 1993); Gregg (pers comm) reported that in studies on salted and air-dried Serrano hams, starting at 4°C and working up to room temperature, the virus survived about six months
- 399 days in Parma hams (McKercher *et al* 1987)
- 112 days in Iberian pork loins (Mebus *et al* 1993)
- 30 days in pepperoni and salami sausage (McKercher *et al* 1978).

Hams from acutely-infected animals should be safe if prepared using York or Parma procedures and heated to 69°C for 3.5 hours or 70-75°C for 30 minutes. Smoked and spiced sausages or air-dried hams require smoking to 32-49°C for 12 hours and drying for 25-30 days (Plowright *et al.* 1994).

Wieringa-Jelsma *et al.* (2011) demonstrated that both salt (NaCl) and phosphate-supplemented salt treatments of natural sausage casings (based on a gel matrix comprising cells infected with ASFV) effectively inactivated ASFV within 48 hours at all temperatures.

Franke-Whittle *et al.* (2013) state that a composting process at a temperature of 60 degrees C for 2 days should provide inactivation of virus (including virus within bone marrow). Gale (2004) recommends application of a two-stage composting process (i.e. turning/mixing) to ensure that all material reaches the required temperature. Gale (2004) also recommends a two month ban on grazing of any pasture to which this product is applied.

Skins, hides and fibres: In a draft assessment, AFFA (2001) has concluded that unprocessed skins and hides from susceptible animals in countries with ASFV pose a high risk. Given the hardy nature of the virus, it is highly likely to be found on the skin of infected animals. Only fully processed or fully tanned skins (including 'wet whites' and 'wet blues') are considered to pose no quarantine threat, because the pH reached will inactivate the virus. Partially processed skins (either limed or pickled) present a lower risk, but could not be relied on to be free of virus.

Semen/embryos: In a draft report, AQIS (2000) notes that there is little information on the appearance and transmission of ASF in semen. Given the widespread secretion / excretion of the virus from infected animals, infectivity of semen is likely, so the risk posed by semen is considered moderate.

Faeces: Muller (1973) reported that ASFV may survive for 60-100 days in faeces, while Eizenberger (unpublished, cited in Haas *et al* 1995) found that ASFV survived for at least 84 days at 17°C and for 112 days at 4°C in slurry under simulated field conditions. Franke-Whittle *et al.* (2013) states that a slurry of virus containing faeces that is heated at 65°C for 5 minutes will inactivate 99.99% of the virus.

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Aujeszky's disease

Agent:

Family Herpesviridae, subfamily Alphaherpesvirinae

Agent type:

Virus

Persistence and inactivation:

General characteristics: Aujeszky's disease virus is labile in the presence of heat, drying, and UV light. It has a half-life of 7 hours at 37°C, but survives for long periods at <4°C (e.g. up to 46 days in contaminated straw and feeding troughs at -20°C) (Schoenbaum *et al* 1991). Survival is up to 21 days at 30°C, reducing to 93 hours at 40°C and is less than one hour at 50°C (Paluszak *et al.* 2012). Virus is destroyed by heating at 56°C within 30 minutes (Maré 1994). The virus is stable between pH 5-9 but is inactivated rapidly outside this range.

ADV is inactivated by most disinfectants, including sodium hypochlorite 0.5% (seconds), phenolic derivatives 3% (10 minutes), and formaldehyde 0.6% (within one hour), and by lipid solvents such as ethyl ether, acetone, chloroform, and alcohol. It is relatively resistant to sodium hydroxide 0.8% and 1.6% (> 6 hours) (Pensaert & Klugge 1989). The following combination of disinfectants have been shown to be effective at inactivating ADV within 30 mins (Dvorakoba *et al* 2008):

- Incidin Plus (1%) – glukoprotamine 26%
- Lysoformin 3000 (1%) – glutaraldehyde 9.5%, glyoxal 7.5%, didecyldimethylammonium chloride 9.6%
- Mikasept KP (0.2%) – peracetic acid 14%, hydrogen peroxide 21%, acetic acid, water
- Sekusept Forte (2%) – glutaraldehyde 3.75%, formaldehyde 11.1%, alkylbenzyltrimethylammonium chloride 2.7%, glyoxal 12%

Terpstra *et al* 2007 states that rehydration/soaking of contaminated surfaces/implements aids in disinfection of surface-dried virus will remove the virus and transmission by embryo transfer under “natural circumstances” is highly unlikely.

Methylene blue light treatment has been shown to inactivate the virus on human osteochondral grafts (Squillace *et al* 2014). However, the survival of virus for any period is likely to be very short given the susceptibility of the virus to drying and UV light. In a draft report, AFFA (2001b) has determined that “the mode of transmission of the disease is such that hides and skins are unlikely to be a significant risk factor in the transmission [of Aujeszky's disease]”.

Van Engelenberg *et al* (2002) demonstrated that a high concentration alcohol mixture (80% ethanol and 5% isopropanol) inactivated the virus within 20 seconds

ADV is spread via oral and nasal discharges, saliva and semen. Close contact facilitates spread but airborne transmission over long distances may be possible. Ingestion of infected tissues and foetuses may also give rise to infection in pigs, dogs, cats and wildlife (Maré 1994).

Carcases and meat products: Although it can be isolated from the tissues of infected pigs after death (Heard 1980, Pensaert & Klugge 1989), ADV is not considered a high-risk contaminant of pig meat products. It does not appear in the OIE review by Farez & Morley (1997) of

‘potential animal health hazards of pork and pork products’. MacDiarmid and Thompson (1997) noted that sheep or goat meat infected with ADV would only pose a risk if fed uncooked to pigs, as dogs and cats are dead-end hosts.

AFFA (2001a) has reviewed the literature on infectivity of ADV in pig meat, citing several papers reporting the transmission of infection through the consumption of the carcasses of infected animals. ADV was recovered from the carcass muscle of clinically affected pigs after storage at 1-2°C for 72 hours (MacDiarmid 1991) but was inactivated in muscle, lymph node and bone marrow from an artificially infected hindquarter after 35 days at -18°C (Durham *et al* 1980).

Wooley *et al* (1981) showed that ADV was inactivated after 72 hours in fermented edible food wastes treated with *Lactobacillus acidophilus*, when temperatures were between 20-30°C. The virus survived beyond 96 hours at temperatures between 5-10°C. Morrow *et al* (1995) concluded that composting was effective at destroying ADV in pig carcasses, provided that maximum temperatures in the pile exceed 60°C. In the compost pile, despite the lack of the thermophilic phase, the total survival time of the viruses ranged from 34 to 44.5 hour (with the highest recorded temperature being 48°C) (Paluszak *et al* 2012)

Milk and milk products: This review did not uncover any specific reports on the spread of ADV via milk, confirming the risk assessment of AQIS (1999a).

Skins, hides and fibres: This review did not uncover any specific reports on the persistence of ADV on skins, hides or fibres. The risk of ADV being present will remove the virus and transmission by embryo transfer under “natural circumstances” is highly unlikely.

Faeces: Several authors have studied the survival of ADV in pig and cattle slurry. Muller (1973) reported that ADV may survive for 3-15 weeks. Turner *et al* (2000) showed that ADV is heat inactivated in slurry within 3 mins at 62°C. Bøtner (1991) reported findings reasonably consistent with previous studies. Inactivation increased with temperature, requiring 15 weeks at 5°C, 2 weeks at 20°C, 5 hours at 35°C and 10 minutes at 55°C (all at pH 7.3-7.9). The virus was inactivated more quickly in cattle than pig slurry (40 minutes compared to 2.5 hours at 40°C) for unidentified reasons.

Semen/embryos: ADV has been detected in the semen of boars and coital transmission is possible (Maré 1994). In a draft report, AQIS (2000) have identified porcine semen as a high-risk quarantine threat for ADV.

AUSVETPLAN (DPIE 1996) notes that whilst ADV can attach to embryos (Bolin *et al* 1982), trypsin treatment will remove the virus and transmission by embryo transfer under “natural circumstances” is highly unlikely.

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Borna disease

Agent:

Unclassified enveloped RNA virus

Agent type:

Virus

Persistence and inactivation:

General characteristics: There are many gaps in the knowledge about Borna disease and its causative virus. Transmission is not well understood. BDV RNA has been detected in nasal discharge, conjunctival fluid and saliva of horses (Richt *et al* 1993), but several attempts to demonstrate infectivity in horse secretions have failed (Staeheli *et al* 2000). Infective virus has been found in the urine of newborn infected rats (Morales *et al* 1988). Infection is thought to be through ingestion or inhalation. BDV is sensitive to lipid solvents and UV light (Richt *et al* 1994).

According to Richt (pers comm), no work has been done on persistence of BDV in carcasses or animal products. No information was discovered in the literature review.

Carcases and meat products: Igata-Yi *et al* (1996), investigating a proposed association between BDV and psychiatric disorders in Japanese people, were unable to find a link between the consumption of raw horse meat and the presence of BDV. AQIS (1999) considered that the transmission of Borna disease by meat or meat products appeared unlikely.

Skins, hides and fibres: No information on this topic was discovered during the conduct of this review. In a draft assessment, AFFA (2001) has determined that the likelihood of transmission of Borna disease via skins or hides is negligible.

Semen/embryos: No information on this topic was discovered during the conduct of this review.

Faeces: Muller (1973), using artificial inoculation, reported that Borna disease virus may survive for 22 days in faeces. No other information on this topic was found.

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Classical swine fever

Agent:

Family Flaviviridae, genus pestivirus

Agent type:

Virus

Persistence and inactivation:

General characteristics: The survival and inactivation of classical swine fever virus has been comprehensively reviewed. It can be described as moderately sensitive in the environment, but persistent under the right conditions (cool, moist, and protein-rich), where it may survive for up to a few weeks. It is rapidly inactivated in the presence of UV light (Edwards 2000). OIE (2016) states that virus is sensitive to drying and ultraviolet light. Virus survives cold conditions (up to 4 weeks in pens in winter). Survival decreases to 7–15 days at 37°C and 3 days at 50°C.

Thermal stability of CSFV varies with the media used, the pH and the strain of virus (Depner *et al* 1992, Edwards 2000). The virus is relatively labile at higher temperatures. Harkness (1985) reported no loss of titre in cell culture fluid after 180 days at 4°C, -30°C, or -80°C, but inactivated the virus after 30 minutes at 56°C or 10 minutes at 60°C. Inactivation took one minute at 90°C, two minutes at 80°C and five minutes at 70°C in a study by Ur Rehman (1987). Other studies have shown longer inactivation times – for example, where defibrinated blood was the medium used. CSFV is susceptible to rapid changes in temperature such as thawing and refreezing (Farez and Morley 1997).

OIE (2016) states the virus to be inactivated by heating meat to 65.5°C for 30 minutes or 71°C for one minute.

Botner *et al* (2012) states that 14 days were required for CSFV inactivation at 20°C and >42 days at 5°C. Virus survival is influenced by the medium in which it is present. CSFV can survive in pig slurry in winter (anaerobic conditions, at 5°C) for longer than 6 weeks. At 20°C, CSFV infectivity declined more rapidly. Most infectivity was lost within 6 days and virus was undetectable by 15 days within slurry and after 4 weeks in EMEM (Eagle's minimum essential medium).

Weesendorp (2008) assessed survival times of CSFV in faeces and urine and determined average half-life values were between 2 and 4 days at 5°C and between 1 and 3 hours at 30°C. Significant differences were observed in survival between virus strains in faeces, however, this was not the case in urine.

Several studies have shown CSFV to be stable at neutral to slightly alkaline values (around pH 5-10). Virus is rapidly inactivated at pH < 3 and >10 (Edwards 2000).

CSFV is an enveloped virus and therefore susceptible to lipid solvents and detergents such as ether, chloroform, and deoxycholate, as well as chlorine-based disinfectants, phenolics, quaternary ammonium compounds, and aldehydes (Edwards 2000). OIE (2016) states that virus is inactivated by chlorine-based disinfectants, cresol (5%), sodium hydroxide (2%), formalin (1%), sodium carbonate (4% anhydrous or 10% crystalline, with 0.1% detergent), ionic and non-ionic detergents, and strong iodophors (1%) in phosphoric acid.

Krug et al (2011) recommends disinfectants be formulated with a minimum of 1000 ppm sodium hypochlorite for disinfection of CSFV dried onto plastic and steel surfaces. Citric acid was shown to be ineffective against CSFV

The principal means of transmission of CSF is from pig to pig, by direct contact or through feeding of infected swill. Infection enters via the oral and intranasal routes and can occur via abraded skin or needle. There is a high level of virus in blood and tissues and saliva. The virus is also excreted in smaller quantities in urine, faeces, nasal and ocular discharges (Terpstra 1994).

Weesendorp et al (2011) looked at the role of excretions and secretions in the spread of CSFV. Three strains of virus with differing virulence were tested and, when contact with blood is excluded, the predicted overall probability of infection was only 0.08 over the entire infectious period. The three strains differed in the relative contribution of secretions and excretions to transmission, although blood had a high probability of causing infection of a susceptible pig when in contact with a pig infected with any strain. This supports the statement that during outbreaks, control measures should ideally be based on the characteristics of the specific virus strain involved, which implies the development of strain-specific measures.

In over 100 cases of CSF managed in Lower Saxony, Germany, between 1993 and 1996, no outbreaks were connected to the transport of carcasses from infected holdings to rendering plants. However, outbreaks occurring within 1km of infected holdings were linked with the movement of rodents after stamping out commenced (Rassow, pers comm).

Ribbens et al (2007) showed that experimentally it is possible to transmit CSF virus through contacts with people, but the risk would depend on several factors

Carcasses and meat products: Pork provides a favourable environment for CSFV. It will survive in pork and processed pork products for months, when meat is chilled, or for years if frozen (Terpstra 1994).

CSFV has been reported to survive longer than four years in frozen pork (Edgar *et al* 1946), and for up to 85 days in chilled fresh pork. Survival in food waste at room temperature may only be several days (Edwards 2000).

Farez and Morley (1997) have thoroughly reviewed survival times of CSFV in various meat products, for example:

- 252 days in Iberian hams
- 140 days in Iberian shoulder and White Serrano hams
- 126 days in Iberian loins (Mebus *et al* 1997)
- one month in the meat, and two months in the bone marrow of salt-cured pork
- 147 days in intestinal casings processed in water at 42.2°C for 30 minutes (Farez and Morley 1997).

Traditional hams with long curing times should be safe (Edwards 2000). CSFV is inactivated in meat by cooking for 30 minutes at 65°C, 15 minutes at 69°C, or one minute at 71°C (Farez and Morley 1997 and Edwards 2000, citing several authors). Heating for 30 minutes at 62.5°C failed to inactivate the virus so temperature control is critical (Edwards 2000). OIE disease card states the virus survives in meat during salt curing and smoking for 17 to >180 days depending on the process used. Virus persists 3–4 days in decomposing organs and 15 days in decomposing blood and bone marrow.

Wijnker et al (2008) demonstrated that the inadvertent spread of CSF virus via porcine sausage casings could be avoided by treating casings with phosphate supplemented salt and storing them for 30 days at temperatures over 4 °C.

Wieringa-Jelsma et al (2011) supported these findings, demonstrating that virus inactivation by phosphate supplemented salt was observed at all temperatures (4, 12, 20 and 25 °C for the entire period of incubation (30 days), except on days 21 and 30 at 25 °C when the non-treated samples had reached the detection limit

Cowan et al (2015) found that at 56 8C, CSFV is inactivated rapidly in muscle, lymph node and fat samples and their data give confidence that composting processes which require a temperature of 60 degC for 2 days, are more than adequate to inactivate CSFV

Gale (2004) looked at a two-barrier composting approach, together with a two-month grazing ban to utilise catering waste on farm land. It can be shown (Haug 1993) that for windrows composting, at least three turns are required to ensure that less than 0.2 percent of the raw material remains in the 'cold' part. For 'in-vessel' composting, the requirement is that over 99.8 per cent of the raw material achieves 60°C for two days.

Skins, hides and fibres: In a draft assessment, AFFA (2001b) has concluded that unprocessed skins and hides from susceptible animals in countries with CSFV pose a high risk. The virus is highly likely to be found on the skin of infected animals. Only fully processed or fully tanned skins (including 'wet whites' and 'wet blues') are considered to pose no quarantine threat, because the pH reached will inactivate the virus. Partially processed skins (either limed or pickled) present a lower risk, but could not be relied on to be free of virus because of its stability over a wide range of pH.

Semen/embryos: AQIS (2000) note that while there are reports of CSFV in semen in the literature, they all seem to originate with a personal communication cited in Thacker *et al* (1984). The virus was apparently isolated from an experimentally-infected boar and was shown to be transmitted to a female. The virus was maintained by freezing.

Maes et al (2016) states that boars experimentally infected with CSF virus have been shown to shed the virus in semen for up to 53 days after infection. Sows that are inseminated with contaminated semen may show seroconversion, the virus may cross the placental barrier causing embryonic mortality, and the virus can be isolated from fetuses.

AQIS (2000) has concluded that excretion of the virus in semen would not be surprising and that the risk of transmission by artificial insemination should therefore be considered moderate.

Faeces: Bøtner (1990, cited in Haas *et al* 1995) found that time to inactivation for CSFV in slurry varied between > 6 weeks at 5°C to instantaneous at 50°C (detection limit 0.7 log₁₀ TCID₅₀/50L). Eizenberger (unpublished, cited in Haas *et al* 1995) found CSFV surviving for at least 70 days at 17°C and for 84 days at 4°C in slurry under simulated field conditions. Have (1984) reported loss of infectivity after about 15 days in liquid slurry.

Terpstra (1994) states that CSFV appears to be inactivated within a few days in manure.

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Infectious bursal disease (hypervirulent form)

Agent:

Family Birnaviridae

Agent type:

Virus

Persistence and inactivation:

General characteristics: Infectious bursal disease virus is highly resistant to environmental influences and difficult to inactivate. It is resistant to freezing and thawing and is stable at pH >2 (Lukert and Saif 1997). Susceptible chickens have succumbed to the disease when introduced to contaminated premises several weeks after depopulation (Geering *et al* 1995). IBDV is resistant to many chemical disinfectants, but susceptible to chloramine solution, formalin and glutaraldehyde (McFerran 1993).

Ferreira *et al* (2010) demonstrated that application of Aviclor (a formulation containing sodium dichloroisocyanurate, acetylsalicylic acid, sodium chloride, and a dye pigment) 500 ppm (0.5 g/L) for 5 minutes effectively inactivated IBDV.

Mandeville *et al* (2000) studied the heat lability of five strains of IBDV, both *in vitro* (Dulbecco's Modified Eagle Medium with 2% foetal calf serum) and by artificial inoculation of chicken products. The *in vitro* study showed one, two, and three log reductions after heating for one minute at 65°C, 71°C and 100°C respectively, with the greatest reduction occurring between 65°C and 77°C. A similar thermal inactivation curve was observed after heating at 71°C or 74°C for up to six minutes. The five strains showed similar characteristics.

Other heating studies have shown:

- apparent neutralisation after 30 minutes at 70°C (Landgraf *et al* 1967)
- little loss of infectivity after five hours at 56°C (Benton *et al* 1967)
- little loss of infectivity after 90 minutes at 60°C (Cho and Edgar 1969)
- reduction in infectivity of bursal homogenate supernatant by one log after 18.8 minutes at 70°C, after 11.4 minutes at 75°C, and after three minutes at 80°C (Alexander and Chettle 1998).

Based on work conducted on behalf of AQIS on IBDV inactivation in chicken meat, fat, skin and bursa supernatant, imported chicken meat is required to be cooked for 165 minutes at 74°C or for 125 minutes at 80°C (AFFA 2000).

IBDV is shed in the faeces for up to two weeks following infection, and is transmitted through oral infection or inhalation (Geering *et al* 1995, van den Berg 2000). Spread occurs with direct contact or via fomites. Vertical transmission has not been demonstrated (Geering *et al* 1995). There are two viraemic stages, the second leading to replication of the virus in several organs, disease and often death (van den Berg 2000).

Crespo *et al* (2016) demonstrated that composting IBDV contaminated litter (using the aerated static pile method) and maintaining the pile at above 55°C for 4 weeks decontaminated the compost.

Guan *et al* (2010) looked at the effect of composting infected chicken carcasses and contaminated litter. This paper demonstrated that after 14 days of composting, with the temperature having been above 55°C for 8.8 of these days, the virus was inactivated in all

specimens. The results suggest that composting of poultry carcasses and manure would help to break the cycle of infection with IBDV.

Gay et al (2010) investigated the use of metam-sodium to inactivate the IBDV in contaminated chicken litter. They demonstrated that IBDV was not inactivated with the lowest amount of metam-sodium, but at higher concentrations the virus was inactivated within 1 hour after application. The results show that metam-sodium can penetrate chicken litter and inactivate enveloped as well as nonenveloped viruses because of its ability to form the active compound methyl isothiocyanate, which acts as a fumigant.

Guan et al (2014) looked at the effectiveness of chemical disinfectants (bleach, Surface Decontamination Foam (SDF), and Virkon) against IBDV at temperatures below 0°C. To simulate the effectiveness of these disinfectants under the influence of organic load (eg farm vehicles and equipment), preparations containing light and heavy levels of organic matter were utilised. At both 23°C and 4°C, Virkon and SDF effectively reduced infectivity within 15 minutes. Bleach, however, required 2 hours at these temperatures to reduce infectivity to the same degree. At -20°C, it required 2 hours for Virkon and 24 hours for SDF to reduce IBDV infectivity in samples with high organic matter content. Under the same conditions, bleach was unable to reduce infectivity in the presence of heavy organic contamination.

Ota et al (2016) looked at the efficacy of calcinated egg shell (Egg-CaO) as a biosecurity enhancement agent in both a powdered and aqueous form. Egg-CaO powder was found to inactivate IBDV, even in the presence of organic matter, within 3 minutes.

Thammakarn et al (2015) assessed the use of bioceramic powder (BCX), at pH 13.0, derived from chicken faeces for its efficacy to inactivate virus and inhibit virus horizontal transmission by the faecal–oral route. They determined that BCX had excellent efficacy and would inactivate IBDV within 3 minutes. Treating IBDV contaminated litter in the cage with BCX was assessed as being able to completely prevent transmission of IBDV to newly-hatched sensitive chicks. Further, transmission of IBDV to the sentinel chicks was significantly inhibited by adding BCX to litter and chicken feed. These data suggest that BCX at pH 13 and derived from chicken faeces was very effective at inactivating IBDV. This method was assessed as suitable for application to bedding materials to prevent viral transmission

Carcases and meat products: No references were found on the persistence of IBDV in carcasses.

According to AFFA (2001) and MAF (1999), IBDV has been found in the muscle of chickens between 48 and 96 hours after infection in studies in Britain. It was also found in liver, kidney, bursa, faeces and blood between 24 and 96 hours after infection. Mandeville *et al* (2000) note that the quantity of IBDV present in chicken muscle during infection is not known, and that studies using artificial inoculation must therefore be interpreted with caution.

The only study to be carried out directly on chicken products has been that by Mandeville *et al* (2000). Chicken products seeded with $10^{5.5}$ TCID₅₀/25µl of virus were heated to 71°C or 74°C under simulated cooking conditions and quickly cooled after reaching target temperatures. Infectivity was not destroyed. The authors noted that complete inactivation of IBDV would -require greater than six minutes at 71°C or 74°C. (This makes an interesting comparison with the AQIS requirements that chicken meat be cooked for 165 minutes at 74°C or for 125 minutes at 80°C (AFFA 2000).)

Jackwood et al (2007) demonstrated that gamma irradiation is not an effective intervention to reduce the risk of IBDV introduction via processed poultry.

Eggs and egg products: No references were found on the persistence of IBDV in or on eggs. Vertical transmission is not thought to be a feature of the epidemiology of IBD, but contamination of eggs with faeces is highly likely. Based on other data on environmental survival, potential persistence on eggs is likely to be of the order of two to three months.

Other products: IBDV may survive for more than 60 days in poultry litter (Vindevogel *et al* 1976).

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Transmissible gastroenteritis

Agent:

Family Coronaviridae

Agent type:

Virus

Persistence and inactivation:

General characteristics: Transmissible gastroenteritis virus (TGEV) is stable at -20°C, able to survive for months at 4-5°C, but loses infectivity after 4 days at 37°C (Harada *et al* 1968, Pensaert 1989). It survives down to pH 3 and is moderately sensitive to trypsin (Pensaert and Callebaut 1994).

TGEV can retain infectivity in the environment for up to three days (Geering *et al* 1995) or “no longer than a few weeks” (Radostits *et al* 2000). The virus is photosensitive (Radostits *et al* 2000).

Casanova *et al* (2009) showed that TGE virus survived and remained infectious in water at both low (4°C) and ambient (25°C) temperatures for days to weeks. At 25°C, time required for 99% inactivation of TGEV in reagent-grade water was 22 days. In pasteurized settled sewage, time for 99% inactivation was 9 days and at 4°C there was less than one log₁₀ infectivity decrease after four weeks.

Casanova *et al* (2010) used TGEV as a model for the severe acute respiratory syndrome (SARS) coronavirus (SARS-CoV) to study the effect of air temperature and relative humidity on virus survival rates. Results showed that when many viruses are deposited, sufficient TGEV may survive for days on surfaces at temperature and relative humidity ranges that are typical of indoor environments.

TGEV is sensitive to lipid solvents and detergents and to a wide range of disinfectants, including sodium hypochlorite, sodium hydroxide, formaldehyde solution (including vapour), iodine, phenolic and quaternary ammonium compounds (Brown 1981).

Goyal *et al* (2014) demonstrated that hydrogen peroxide vapour was virucidal (>4 log₁₀ reduction) against TGEV type 1 that dried onto stainless steel surfaces (including at the lowest vapourised volume tested of 25ml).

Hulkower *et al* (2011) looked at the effect of several disinfectants on TGEV inactivation. After 1-minute contact time there was a log₁₀ reduction of: 3.2 for 70% ethanol; 2.0 for phenolic compounds; 2.3 for OPA (ortho-phthalaldehyde); 0.35 for 1:100 hypochlorite; 4.0 for 62% ethanol; and 3.5 for 71% ethanol. Ethanol was the most and hypochlorite the least ineffective chemicals against TGEV.

TGEV is highly contagious. It is principally spread by the faecal-oral route. While several authors note that transmission may take place via aerosols (e.g. Forman 1991), Pensaert and Callebaut (1994) state that spread of the virus by this means is not known to occur. The virus replicates in the respiratory tract and is present in aerosols, but in lower concentrations than in faeces. Spread is by direct contact or indirect through the environment (Geering *et al* 1995, Pensaert and Callebaut 1994). Virus has also been found in the milk of infected sows (Kemeny *et al* 1975).

Pigs are the only species affected, and although dogs, cats and foxes have been infected experimentally, it is unlikely they play a role in the epidemiology (Pensaert and Callebaut 1994).

Carcases and meat products: TGEV has been found in a range of tissues. Cook *et al* (1991) cite an early study in which TGEV was transmitted by homogenates of kidney, spleen, liver, lungs, brain, and gastrointestinal tract (Bay *et al* 1949), and another in which virus was isolated in nasal and tracheal mucosa, oesophagus, lung, intestine, and lymph nodes (Harada *et al* 1969).

Forman (1991) succeeded in transmitting infection by feeding ground-up samples of muscle, bone marrow and lymph node from recently infected pigs to one- and three-week-old piglets. The tissues had been stored at -25°C for at least 30 days. The conditions were intended to mimic real abattoir conditions. The author concluded that the risk of transmission by this means was low but real.

In a similar study, Cook demonstrated transmission from apparently healthy animals from a TGE-endemic area (Cook *et al* 1991). TGEV was found in tonsils from 4 of 500 pigs but not from lymph nodes or muscle. The study showed the risk of introducing TGEV through pig carcasses from TGE-endemic areas even when they have passed ante-mortem examination.

The acid stability of TGEV means it is not inactivated by rigor mortis or lactic fermentation of certain meat products (Harada *et al* 1968, Leman *et al* 1986). Putrefaction is known to destroy the virus (Leman *et al* 1986). Sausage casings are considered unlikely to transmit TGE because they are salted and stored at room temperature, although no direct evidence could be found for this (AQIS 1999). References on the effect of cooking or curing on the TGE virus were not found (see also AFFA 2001a), although AUSVETPLAN (DPIE 1996) states that TGEV will be destroyed by cooking.

Skins, hides and fibres: No specific reports were found on this aspect of TGEV. In a draft assessment, AFFA (2001b) have concluded that skins and hides pose an insignificant risk in the transmission of TGEV.

Semen/embryos: There are no reports of TGEV in the semen of boars, or spread of the disease via artificial insemination. As the virus is excreted in the faeces, there is some risk of semen contamination from this source, but this risk is low (AQIS 2000).

Faeces: TGEV is excreted in the faeces for around 14 days (Pensaert *et al* 1970). Faeces containing the virus was no longer infective after 10 days at 21°C (Young *et al* 1955). Faeces containing 10⁵ TGEV was inactivated within 6 hours when exposed to direct sunlight (DPIE 1996).

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Vesicular exanthema

Agent:

Family Caliciviridae

Agent type:

Virus

Persistence and inactivation:

General characteristics: Vesicular exanthema is relatively resistant to environmental influences. It may survive for up to 2 years at refrigerator temperatures, and up to 6 weeks at room temperature (Madin 1989). It is inactivated after 60 minutes at 62°C or 30 minutes at 64°C, and outside pH range 3 to 9 (MacDiarmid 1991).

According to Madin (1989), farms that are heavily contaminated must be regarded as infectious for several months unless there is vigorous disinfection. However, Bankowski (1965) reported the case of a farm on which there was no evidence of infectivity seven days after depopulation.

VESV is susceptible to most common disinfectants, but not detergents (DPIE 1996). Madin (1989) specifically nominates VESV sensitivity to 2% sodium hydroxide, 0.1% sodium hypochlorite, and 2% citric acid. Manure, fat and other organic matter is protective and must be removed prior to disinfection (DPIE 1996).

VESV is excreted in the saliva and faeces of infected pigs, from about 12 hours prior to clinical signs and for 1-5 days after that (Wilder and Dardiri 1978). Vesicles containing virus also rupture. Transmission can be by direct, but not indirect, contact between pigs, and via the ingestion of untreated swill, but whether the infection establishes via the oral route or via skin is unknown. Infection has been established through scarified skin. VESV is found in a wide range of tissues of infected pigs (Bankowski 1965, Thomson 1994).

Carcases and meat products: Viral particles can be identified in the muscle of slaughtered pigs during the viraemic period (Wilder and Dardiri 1978). Patterson and Songer (1954) showed that feeding infected muscle tissue, lymph node, heart muscle, spleen, lung, kidney, blood and crushed bone to susceptible pigs can cause infection. The US was apparently unable to eradicate VES until cooking garbage before feeding to swine became mandatory (Madin 1989).

Mott *et al* (1953) demonstrated survival of VESV in meat scraps of up to 4 weeks at 7°C, and at for 18 weeks at -70°C.

Madin (1989, citing Traum and White unpublished 1941) noted that cooking meat at 184°F (84.5°C) under 10lb (4.5kg) pressure did not destroy infectivity. However, MacDiarmid (1991) reported that meat would be safe after 2-3 minutes at 80-100°C or 25 minutes at >70°C.

Radostits *et al* (2000) state that eradication should involve the slaughter of infected animals, but that carcasses can be salvaged for human consumption provided they are treated to inactivate the virus.

Skins, hides and fibres: No specific reports were found on this aspect of VES (see also AFFA 2001b).

Semen/embryos: Vesicular exanthema has been reported in boar semen (Cartwright and Huck 1967), but it is thought unlikely that the disease could be spread by artificial insemination (Hare 1985). Transmission via semen is not noted in any of the reviews examined during this study (Geering 1995, Madin 1989, Radostits 2000, Thomson 1994). AQIS (2000) has concluded that the risk of transmission of VES via artificial insemination is low.

Faeces: Virus is found in the faeces of infected pigs (Wilder and Dardiri 1978). No specific reports were found on its persistence or inactivation in faeces.

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Prions

Bovine spongiform encephalopathy and scrapie

Agent:

BSE and scrapie agents

Agent type:

Prion

Persistence and inactivation:

General characteristics: BSE agent has so far only been found only in CNS tissue, distal ileum and bone marrow. However, the much wider distribution of agent in kuru, Creutzfeld-Jakob disease and scrapie suggests that all tissues should be treated with caution (Brown 1998).

The concentration of infective agent in TSEs is extremely high in naturally occurring disease. The normal, conservative estimate used for oral infective dose (ID₅₀) of BSE is 0.1g infected CNS tissue for cattle, 1g for humans. Bovine brain and spinal cord weigh approximately 750g, so assuming 1000g for risk assessment purposes, there are 10,000 infective oral doses of BSE per cow (MAFF 2000).

Transmissible spongiform encephalopathy agents are extremely difficult to inactivate. In Iceland, scrapie has been contracted by sheep grazing pastures that had lain unused for three years after having been grazed by scrapie-infected sheep (Palsson 1979). Booth et al (2013) referenced a study by Georgsson et al (2006) which presented anecdotal evidence of environmental transmission wherein naïve sheep contracted scrapie following habitation in a farm building occupied by scrapie infected sheep 16 years earlier.

Virtually all conventional disinfection methods have failed to reliably and completely inactivate TSE agents (Taylor 2000), including:

- irradiation and UV light
- dry heat, even to 360°C for one hour (drying of tissue is known to enhance its thermostability)
- autoclaving, although gravity-displacement autoclaving at 132°C for 4.5 hours has been recommended, as has porous-load autoclaving at 134-138°C for 18 minutes
- acids and bases, including hydrochloric acid (pH 0.1) for one hour and 2M sodium hydroxide for two hours, although combinations of sodium hydroxide and heat have been effective (see below)
- alkylating agents, including formalin and glutaraldehyde
- detergents, although boiling in SDS has been reported as effective for fluids
- halogens, except strong sodium hypochlorite (20,000ppm available chlorine for at least one hour)
- organic solvents, including acetone, chloroform, and phenolics
- oxidising agents, including hydrogen peroxide and peracetic acid
- salts, including potassium permanganate
- chaotropes, including urea
- proteolytic enzymes, including trypsin, although pronase and proteinase K may reduce titre significantly over prolonged exposure periods.

Taylor (2000) concluded that disinfection procedures that efficiently and/or rapidly fix proteins (alcohols, aldehydes, rapid steam heating) enhance the thermostability of TSE agents. The only completely effective methods were strong (20,000ppm) sodium hypochlorite solutions, applied for one hour, boiling in 1M sodium hydroxide for at least one minute, or gravity-displacement autoclaving in the presence of sodium hydroxide (e.g. 121°C for 30-60 minutes plus 1M or 2M NaOH).

A review by Booth et al (2013) listed the following effective treatments:

- autoclaving for 4.5 hours at 132°C;
- autoclaving for 90 minutes at 121°C in 1 M NaOH (Prusiner et al., 1984);
- exposure to the denaturing chaotropic salt guanidinium thiocyanate (>6 M) (Prusiner et al., 1993);
- incineration (15 minutes at 1000°C) (Brown et al., 2004);
- exposure to the phenolic disinfectant Environ LpH (1% for 30 minutes) (Ernst and Race, 1993; Race and Raymond, 2004);
- 1-hour exposure to 2% sodium hypochlorite (Kimberlin et al., 1983);
- 2-hour exposure to acidic 1% SDS (pH 4.5) at 65°C following 30-minute exposure to 4% SDS (neutral pH) at 65°C (Peretz et al., 2006).

The same review looked at microbial and enzymatic inactivation of prions in soil environments. It showed that micro-organisms present in animal waste compost and the earthworm gut may be able to work cooperatively to inactivate prions. To date, fungi have not been evaluated for their ability to degrade prion protein (PrPTSE) or reduce TSE infectivity. Several studies have successfully employed purified enzymes for degradation of PrPTSE, but many require the use of pH extrema, high temperatures, or detergents. Enzymes shown to be capable of effecting PrPTSE degradation, reduction in prion infectivity, or both have been identified as serine or serine-like Booth et al (2013).

A review article by Bebermeyer et al (2003) looked at dental practice implications of prion disease. They found best practice for non-disposable instruments involved thorough physical cleaning, soaking in hot 1N sodium hydroxide solution for 1 hour, and then autoclaving in a vacuum or porous-load autoclave at 134°C to 138°C for 18 to 20 minutes.

Heindl et al (2006) examined the effect of high pressure at varying temperatures, on the structure of prion proteins. Their results prove that pressure (over 800 MPa) combined with medium heat (not over 60°C) are enough to cause an irreversible and acceptable inactivation of certain (but not all) fractions of infectious prions. By optimising the pressure conditions, mild inactivation of contaminated materials is possible with this technology. More work is needed that examines effectiveness of various pressure combination with other denaturants besides heat.

Gas plasma sterilisation is a method of sterilisation that shows promise for inactivating prions. The main indication for this method would be sterilisation of fragile medical equipment rather than large scale disinfection (Sakudo et al 2011).

A review by Smith et al (2011) looking at the fate of prions in soil states that 'Preliminary research suggests that serine proteases and the microbial consortia in stimulated soils and compost may partially degrade TSE prion protein. Transition metal oxides in soil (viz. manganese oxide) may also mediate prion inactivation. Overall, the effect of prion attachment to soil particles on its persistence in the environment is not well understood, and additional

study is needed to determine its implications on the environmental transmission of scrapie and chronic wasting disease'

Brown (1998) stated that, regarding BSE-infected materials, "a fairly obvious recommendation, based on the science, would be that all material that is actually or potentially contaminated with BSE, whether whole carcasses, rendered solids, or waste effluents, should be exposed to lye [sodium hydroxide] and thoroughly incinerated under strictly inspected conditions. Another is that the residue is buried in landfills to a depth that would minimise any subsequent animal or human exposure, in areas that would not intersect with any potable water-table source".

Carcasses and meat products: Studies have detected residual scrapie infection in the packaging and adjacent soil around scrapie-infected hamster brains buried for three years. Temperatures at the site ranged from -20°C to 40°C (average 3°C to 25°C), with rainfall of 1000mm. Infectivity was reduced by 98-99%. There was little leaching from the initial site, with no infectivity detected more than 8cm from the burial site (Brown and Gajdusek 1991).

In a series of reports for the UK's Environment Agency, Det Norske Veritas Ltd (Environment Agency 1997a-e) estimated the risks posed to human health via environmental pathways, and of various options for disposal of material potentially contaminated with BSE. The assumptions may now be out of date, and the data used specific to the sites modelled. However, the approach taken provides a useful framework for comparing various disposal options.

In a risk assessment of landfills containing BSE-infected carcasses from prior to 1991, Environment Agency (1997d) concluded that "risk estimates for contamination of water supply with BSE infectivity are all well below any level that would be considered to be of any significance". The calculated risk for the most exposed individual ranged from one in 10,000 million years to one in one million years³. The greatest risk came from leachate contaminating water supplies. A favourable factor is that prions tend not to move with leachate, instead tending to stick to other proteinaceous material or solids (consistent with Brown and Gajdusek 1991).

The risk assessment on disposal of BSE-infected carcasses in incinerators estimated a maximum risk of individual human infection of 1 in 1000 million (Environment Agency 1997c). Residual infectivity after incineration was assumed to be 0.002%.

Risk assessments have also been conducted for treated waste water from plants rendering cattle from the Over Thirty Months Scheme (Environment Agency 1997a, MAFF 2000) and for burning rendered products from the OTMS in power stations (Environment Agency 1997b). An overview of public health risks from BSE via environmental pathways in the UK has also been published (Environment Agency 1997e).

The policy in the UK is to dispose of BSE-infected tissues by incineration. Landfill has not been used for disposal since 1991 (DEFRA 2001). The UK currently has about 200,000 tons of ash left from incinerated carcasses from the foot-and-mouth eradication campaign. The pyres reached high temperatures (evidenced by the fragmentation of bones) but there is concern that the animals slaughtered could potentially include pre-clinical cases of BSE. The ash will therefore be incinerated as a precaution (Jeffrey pers comm).

³ The risks quoted by the Environment Agency have been put into perspective by Gunn (2001), who noted that the probability of dying from cancer was one in 300, and from being involved in a railway accident one in 500,000.

Milk and milk products: No reports were found of scrapie or BSE agents being present in milk. AQIS (1999) has accepted the conclusions of the OIE that scrapie and BSE are not transmitted by milk.

Skins, hides and fibres: No reports were found of scrapie or BSE agents contaminating skins, hides or fibres. The agents would not be expected to be present on these products. In a draft assessment, AFFA (2001) has concluded that skins, hides and fibres pose no quarantine hazard for the introduction of scrapie or BSE.

Semen/embryos: The risk of transmission of BSE and scrapie via semen and embryos has been reviewed by Wrathall (1997). Several studies have been unable to detect scrapie agent in ram semen or to demonstrate venereal transmission from infected rams (Palmer 1959, Foote unpublished cited in Wrathall 1997), but these studies had shortcomings and further work is needed. There appears to be no published work on scrapie in goat semen. Transmission of BSE via semen has not been observed in either experimental conditions or by analysis of mating records in Britain, and the risk is thought to be small or non-existent (Wrathall 1997).

The literature on possible transmission of TSEs via embryos is more complicated. Again, there are shortcomings in published studies on scrapie in sheep and goats from groups in the USA and Scotland (see for example Foote *et al* 1993, Foster *et al* 1996), and it is still not possible to say whether scrapie is transmitted via embryos. A study by Foster *et al* (1999) failed to transmit experimental BSE in goats via embryos. A large trial on transmission of BSE by embryo transfer in cows in the UK had shown no transmission of the agent more than five years after the first transfers (Wrathall 1997).

Faeces: No reports were found of scrapie or BSE agents being present in faeces.

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Bacteria, mycoplasmas and fungi

Anthrax

Agent:

Bacillus anthracis

Agent type:

Bacterium

Persistence and inactivation:

General characteristics: *Bacillus anthracis* is an aerobic, spore-forming bacillus. It is normally transmitted by the ingestion of spores, but infection can also take place via wounds and insect bites. Infection through inhalation is thought to be rare (de Vos 1994), and windborne spread of spores is minimal (Turnbull *et al* 1998).

Entry of *B. anthracis* to the body is followed by replication in the regional lymph nodes, septicaemia, and invasion of all body tissues (Radostits *et al* 2000).

Spores are never found in the living body. Sporulation is a response to nutrient depletion from drying of tissues and aerosolisation of fluids, with the opening of the carcase allowing 'escape' to an aerobic environment (Dragon and Rennie 1995). Sporulation is inhibited by high partial pressure of CO₂ and temperatures below 20°C, and only takes place under appropriate conditions, most notably when an infected carcase is opened. Germination of spores occurs at 20-40°C and at relative humidity greater than 80% (de Vos 1994).

The vegetative (growing) form of *B. anthracis* is relatively labile. It is killed by 60°C dry heat for 30 minutes. Spores are much more robust, requiring 140°C dry heat for up to 3 hours for inactivation (Buxton and Fraser 1977). Moist heat destroys spores at 100-115°C after 14.2 minutes (Bohm 1990).

Anthrax spores are generally resistant to alcohols, phenols, quaternary ammonium compounds, ionic or non-ionic surfactants, acids and alkalis (Bengis 1997, de Vos 1994). They are susceptible to 10% hot caustic soda, 4% formaldehyde, chlorine-containing disinfectants, 7% hydrogen peroxide, and 2% glutaraldehyde (Whitford 1987). Standard UV treatments (254-nm-wavelength UV) that are effective against *B. subtilis* spores are likely also to effectively inactivate *B. anthracis* spores (Nicholson *et al.* 2003)

De Vos (1994) cites several factors that impact on the survival of survival of anthrax spores in the environment: initial numbers, topography, climate, and the presence of certain chemicals, other microbes and plant material. In soils of high biological activity, survival may only be of the order of 3-4 years (Whitford 1978). Under ideal conditions, spores may survive almost indefinitely in the environment. Infective spores determined to be 200 years old have been found (de Vos 1994).

Carcases and meat products: All parts of the carcase of an anthrax victim may be infective. In the unopened carcase, survival of the organism is short. Estimates include:

- no longer than 3 days at 25-30°C or higher (Stein 1947)
- two weeks in the skin, one week in the bone marrow (Minett 1950)
- up to 4 weeks at temperatures of 5-10°C (Whitford 1978).

Opening of the carcass by scavengers or humans usually triggers sporulation before all the vegetative forms have been inactivated. The concept of survival of anthrax spores in carcasses is almost meaningless, as the spores may persist much longer than the carcass itself. Several factors – scavengers, water, wind – act to disseminate the spores into the environment (Dragon and Rennie 1995).

Early identification and disposal of carcasses prior to opening helps to minimise environmental contamination. Carcasses should be incinerated intact, or buried in a 2-metre deep grave. A detailed review indicates that the use of lime, or any calcium based substance may not only be ineffective against bacterial spores, including anthrax, but may instead facilitate preservation of the spores (Himsworth 2008). The use of lime as an agricultural disinfectant when dealing with anthrax may therefore be contraindicated. The WHO (1994) has noted that burning is the most reliable method of destroying spores when it is correctly done. No specific studies were found of the survival or inactivation of anthrax spores in carcasses after burning.

Bone meal contaminated with anthrax was found to be infective after 15 minutes of steam treatment at 115°C degrees or 3 hours with dry heat at 140°C (De Kock *et al* 1940). WHO guidelines for disinfection of bone meal (Whitford 1987) include several alternatives:

- steam sterilisation at 2.7 bar for 2 hours in a digester (less than 4 tonne capacity)
- benzene vapour at 95-115°C for at least four hours, followed by 2 hours with live steam at 5.4 bar (for broken bones)
- benzene vapour for eight hours at 95-115°C (for broken bones).

Heat sterilisation of bone meal for at least 3 hours should also be effective (Whitford 1978).

Milk and milk products: No specific statements were found in the literature about the excretion of anthrax bacteria in milk. AQIS (1999) states that anthrax is not known to be transmitted in dairy products.

Milk production decreases in lactating cows and the residual milk is either blood-stained or yellow (de Vos 1994). Radostits *et al* (2000) stress the importance of preventing milk from entering the human food chain. WHO guidelines (Whitford 1987) advise that milk should can be considered decontaminated by adding chloride of lime (at least 25% active chlorine) at 1kg/20L of milk for six hours.

Skins, hides and fibres: Numerous outbreaks of anthrax have been traced to infected hides and associated products (de Vos 1994). In fact, de Vos (1994) states that hides from carcasses of infected animals should be regarded as permanently infected with *B. anthracis*.

Whitford (1987) gives details of the WHO guidelines for disinfection of infected materials. Wool should be disinfected with formaldehyde, and hides with a mixture of 2.5% hydrochloric acid and 15% salt. Hair may be boiled, autoclaved at 120°C for 20 minutes, dry heated at 95°C for 24 hours, or steamed for six hours.

AFFA (2001) lists methyl bromide, formaldehyde (4-5%), glutaraldehyde, hydrogen peroxide, peracetic acid and gamma radiation (4 MRad) as being capable of sterilising anthrax spores. Later WHO (1998) guidelines cited by AFFA (2001) also include ethylene oxide. AFFA (2001) notes that no studies could be found to demonstrate efficacy of ETO on its own, but that it was effective in combination with methyl bromide (1:1.44 w/w) after 24 hours at 20-25°C.

De Vos (1994) states that even products made after tanning and curing of hides cannot be considered safe. AQIS (2001) has concluded, however, that the liming/dehairing process of commercial tanning should destroy anthrax spores.

Semen/embryos: No references were found to the presence of anthrax in semen. It seems unlikely that semen would be collected from a bull during the viraemic period.

Faeces: The faeces of infected animals may contain anthrax spores. De Vos (1994) advises disposal of excreta by incineration or by burial in a 2-metre deep hole, covered with one part of chloride (of at least 25% active chlorine) to three parts soil prior to filling in the hole. Note previous comments regarding role of calcium-based disinfection (ie lime) as a potential spore preservative.

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Heartwater

Agent:

Erlischia ruminantium

Agent type:

Rickettsia

Persistence and inactivation:

Erlischia ruminantium is a rickettsia. It is an obligate intracellular parasite, infecting endothelial cells of blood vessels of various organs, and is seen in the blood in association with red blood cells, neutrophils and plasma. *E. ruminantium* is transmitted between vertebrate hosts by ticks of the genus *Amblyomma* (Bezuidenhout *et al* 1994). Heartwater is not contagious, and even experimental transmission by needle is difficult (Uilenberg pers comm).

The only literature of relevance pertains to work attempting to preserve the organism for experimental purposes. Such work has generally shown that *E. ruminantium* is heat labile and loses its viability within 12-38 hours at room temperature (Uilenberg pers comm). Alexander (1931) could maintain *E. ruminantium* in defibrinated blood for up to 38 hours. He observed that *E. ruminantium* generally did not survive for more than 24 hours at room temperature, and in some cases lost viability within 12 hours.

In one exceptional case, infectivity was maintained in blood stored at room temperature for 4 days (Henning 1956). Alexander (1931) noted that low temperatures seemed to favour the viability of *E. ruminantium*. Infected ovine spleen and blood homogenates kept at -76°C maintained infective virus for at least 2 years (Neitz 1968). Logan (1987) reported effective preservation of *E. ruminantium* at -70°C to -196°C for indefinite periods of time in a variety of organ suspensions. Blood collected from infected goats and stored for as long as 72 hours at 4 degrees were still infectious to mice.

No reports were found on the survival of *Erlischia ruminantium* in carcasses or animal products. As Uilenberg (pers comm) has pointed out, this is partly due to the extreme difficulty in proving if the organism is alive.

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Brucellosis (*B. abortus*)

Agent:

Brucella abortus

Agent type:

Bacterium

Persistence and inactivation:

General characteristics: Huddleson (1943) reported that *Brucella abortus* may survive in the environment for:

- up to eight months in aborted foetuses (in the shade)
- 2-3 months in wet soil
- 1-2 months in dry soil
- 3-4 months in faeces.

AFFA (2001) quotes a survival time for *B. abortus* of up to three weeks in the environment in moist, humid conditions. Radostits *et al* (2000) describe infectivity as persisting for up to 100 days in winter and up to 30 days in summer in temperate climates. Aune *et al* (2012) states *Brucella* bacteria can persist in foetal tissues and in soil or vegetation for 21–81 days depending on month, temperature, and exposure to sunlight. *B. abortus* has been found to be quite resistant to a decrease in pH (Davies and Casey 1973), but sensitive to heat, sunlight, and standard disinfectants, including phenolics, halogens, quaternary ammonium compounds, and aldehydes at 0.5-1.0%. The bacterium can be maintained indefinitely by freezing (Radostits *et al* 2000).

A review by Franke-Whittle (2013) states that although no literature reports regarding the survival of *Brucella* after a process of alkaline hydrolysis (AH) could be found based on the report of Kaye *et al.* (1998), it could be expected that AH would be capable of the inactivation of *Brucella* species

B. abortus is excreted in greatest quantities in uterine discharges, abortions and foetal membranes, and in milk. Urine, faeces and semen also contain the bacterium, as do hygromas caused by the infection, but these sources are less important in the epidemiology of the disease. Entry to the body is through ingestion, inhalation, via the conjunctiva, skin abrasions or intact skin, and congenitally. Transmission is by direct contact or via the environment (Bishop *et al* 1994).

Carcases and meat products: *B. abortus* can be isolated from many organs of infected cattle, creating a zoonotic risk for people handling carcasses (Radostits *et al* 2000). Bishop *et al* (1994) recommends incineration of foetuses, placenta and discharges from infected animals.

Apart from Huddleson's (1943) report of persistence of *B. abortus* in aborted foetuses for up to eight months in the shade, no references were found on the persistence or inactivation of in carcasses or meat products. AQIS (1999a) lists bovine brucellosis among those diseases "unable to be transmitted via meat or meat products".

Milk and milk products: Infected animals may shed *B. abortus* in colostrum and milk intermittently throughout the lactation period. Unpasteurised milk has been responsible for human infections (Bishop *et al* 1994).

Brucella spp are destroyed by pasteurisation (AQIS 1999b, Davies and Casey 1973, Keogh 1971). Heat treatment to 'thermise' milk for cheese production (62°C for 15 seconds) is not

sufficient to inactivate *Brucella* (AQIS 1999b). Davies and Casey (1973) inactivated *B. abortus* in milk by heating for 15 seconds at 71.7°C, and at all time / temperature combinations down to 5 seconds / 65°C. Survival in whey was less than 4 days at 17-24°C (associated with a sharp decline in pH from 5.9 to 4.0), but at least 6 days at 5°C in whey (where pH dropped only to 5.4). When *B. abortus* was stored in a citrate/phosphate buffer solution, viable organisms were found after eight days at pH 4.0, while at pH <4.0 all bacteria died within 78 hours.

It is well documented that *B. abortus* survives the ordinary cheese making process and persists for long periods in various cheese types. However, human infection from contaminated cheese is rare (Keogh 1971).

Falenski et al (2011) states that after inoculation with bacteria, under normal storage conditions *Brucella* survived in UHT milk for 87 days, for 60 days in water and less than a week in yoghurt. Also, when milk was inoculated with low bacterial numbers, *Brucella* multiplied by five log units within three weeks

Skins, hides and fibres: Contamination of these products may occur but in a draft assessment, AFFA (2001) has determined that they do not pose a quarantine risk for *B. abortus*.

Semen/embryos: *Brucella abortus* can be found in the testicles, seminal vesicles and semen of infected bulls (Radostits *et al* 2000). AQIS (1999c) states that *B. abortus* can survive freezing in semen and that it can be transmitted by artificial insemination.

B. abortus is likely to be collected with uterine flushes containing embryos (AQIS 1999c). Six washes will generally ensure the removal of the bacteria from the embryos (Stringfellow *et al* 1984), unless the zona pellucida is damaged (Stringfellow *et al* 1986). Embryo transfer probably does not present a significant risk in the transmission of *B. abortus* (Campo *et al* 1987).

Faeces: *B. abortus* is excreted in the faeces (Bishop *et al* 1994), and additional contamination of faeces is likely from vaginal discharge (Vergier 1980).

It has been found that increasing the temperature of storage decreased the survival time of *Brucella* spp in manure. Reported survival times include:

- 385 days at 8°C
- at least 8 months at 12°C
- 29 days at 25°C
- less than one day at 37°C
- less than 4 hours at around 70°C

(King 1957, Kuzdas and Morse 1954, Plommet 1977, Vergier 1980).

Vergier (1980) concluded that composting of bedding, faeces and urine would inactivate *Brucella* within an hour. The organism would however survive long periods in a slurry and would be a source of further infection unless treated with xylene 1000ppm.

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Brucellosis (*B. melitensis*)

Agent:

Brucella melitensis

Agent type:

Bacterium

Persistence and inactivation:

General characteristics: Survival of *Brucella melitensis* in the environment is like that of *B. abortus* (Herr 1994). *B. abortus* has been reported to last up to several months in the environment, longer in moist, cool conditions out of direct sunlight (see Bishop *et al* 1994, Radostits *et al* 2000). Geering *et al* (1995) state that *B. melitensis* can survive for up to three months in soil protected from sunlight and up to six months in necrotic placenta and foetus. Gilbert *et al* (2012) showed that *B. melitensis* survived in water at 25°C for 2 days and at 5°C for up to 2 weeks.

According to Mitscherlich and Marth (1984), *B. melitensis* is rapidly inactivated by moist heat and outside pH range 5-8. The organism was inactivated at:

- between 7.5 and 10 minutes at 60°C
- between 5 and 7.5 minutes at 61.1°C
- less than 5 minutes at 62.8°C.

El-Daher *et al* (1990) found that in broth, *B. melitensis* survived for:

- more than 4 weeks at pH 5.5 or above
- less than three weeks at pH 5
- one day at pH 4

and did not survive below pH 4.

Al-Marir (2008) demonstrated susceptibility of *B. melitensis* to UVC radiation at 254 nm. At an intensity of 18.7 mW/cm² of UVC, the time required for inactivation of *B. melitensis* was 240 seconds in both dark and light, whereas it was 120 seconds and 240 seconds in dark and light respectively at an intensity of 19.5mW/cm²

Bleichert *et al* (2014) demonstrated that *B. melitensis* was inactivated upon contact with copper surfaces within 3 minutes.

After an initial bacteraemia, *B. melitensis* localises in lymph nodes, udder and uterus or testes. Infected females shed large quantities of bacteria in genital discharges and abortions. These provide the main source of infection, which takes place principally via inhalation, but also via abraded skin in goats and sheep. Shedding also occurs in milk, urine and semen. Ingestion of infected milk or meat is a common means of transmission to humans (Garin-Bastuji *et al* 1998, Geering *et al* 1995, Herr 1994).

Carcases and meat products: No specific references were found on the survival of *B. melitensis* in carcasses or meat products. *B. abortus* has been shown to survive up to 44 days in guinea pig carcasses in cold conditions (Timoney *et al* 1988). *B. melitensis* can survive for up to six months in necrotic foetal and placental material (Geering *et al* 1995). Alton (1987) recommended that all membranes and aborted foetuses should be incinerated where possible and if not buried deeply.

Humans have become infected with *B. suis* by ingestion of uncooked meat or bone marrow from infected animals (Acha and Szyfres 1987). MacDiarmid and Thompson (1997) note that infection could theoretically be picked up from infected carcasses of sheep or goats by dogs or pigs, but that pigs are dead-end hosts and transmission from dogs to other animals is rare.

Milk and milk products: *B. melitensis* is excreted in the milk of infected sheep and goats (Herr 1994) and milk products have been implicated by several authors as the source of human disease (e.g. Thapar and Young 1986).

Brucella spp are destroyed by pasteurisation (AQIS 1999, Davies and Casey 1973, Keogh 1971). El-Daher *et al* (1990) found that survival of *B. melitensis* in dairy products was inversely proportional to pH. They concluded that soft cheese was the most likely of the products examined to present an infective risk, because it took up to 72 hours to fall below pH 4 and had the highest bacterial counts at 48 hours. Yoghurt posed an intermediate risk, while bacteria were not found in any of the milk samples by 24 hours. *B. melitensis* did not survive for even four hours in buttermilk, which had an initial pH below 4.

In a review of the literature, Rammel (1967) noted that *B. melitensis* had been found in Feta after 4-16 days and up to 90 days in Pecorino cheeses.

Falenski *et al* (2011) states that under normal storage conditions *Brucella* sp survived in UHT milk for 87 days, for 60 days in water and less than a week in yoghurt

Skins, hides and fibres: No specific references were found on this aspect of the disease in the literature. Contamination of these products with genital discharges could be expected to occur. In a draft assessment, AFFA (2001) has concluded that skins, hides and fibres could carry *Brucella* for up to three months and that unprocessed product from endemic areas posed some quarantine risk. Normal processing methods of alkaline scouring, acid pickling or liming / dehairing should eliminate this risk in processed or partially-processed skins, hides or fibres.

Semen/embryos: *B. melitensis* is commonly shed in semen, although this seems only to have been confirmed recently (Garin-Bastuji *et al* 1998). No reports were found of transmission of *B. melitensis* from goat or sheep semen, nor of the infection of embryos by the agent. However, AQIS (2000) has concluded that the quarantine risk posed by semen and embryos for *B. melitensis* is high.

Faeces: Excretion of *B. melitensis* in faeces is not described in texts on the disease, although *B. abortus* is shed by this route (Bishop *et al* 1994). However, faeces are highly likely to become contaminated by the heavy load of organisms in vaginal discharges after parturition or abortion (Verger 1980).

Survival characteristics of *B. melitensis* in faeces are likely to be similar to those of *B. abortus*. Verger (1980) has shown that composting (to reach 70°C) of faeces, urine and straw bedding will kill *Brucella* species within one hour. Studies showing long term survival of *B. abortus* in faecal slurry in storage pits suggest that *B. melitensis* would show good viability in these conditions. Xylene 1000ppm is an effective disinfectant for *B. abortus* (Verger 1980).

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Bovine tuberculosis

Agent:

Mycobacterium bovis

Agent type:

Bacterium

Persistence and inactivation:

General characteristics: *Mycobacterium bovis* is relatively resistant to heat, desiccation, and disinfectants (Radostits *et al* 2000). Heat treatments (hot air, burning, cooking, pasteurisation, pressurised steam) are the most useful methods for inactivating *M. bovis*.

M. bovis is relatively resistant to chemical disinfectants because of its waxy, hydrophobic cell wall (Russell 1996). Inorganic acids, alkalis, quaternary ammonium compounds and chlorides are ineffective. Formalin (3%), lysol (2%), phenol (2.5%), activated chloramine (1-3%), cresols and iodophors are effective (Huchzermeyer *et al* 1994).

Several studies have been conducted on the survival of *M. bovis* in the environment, and the findings are reasonably consistent.

An Australian study demonstrated survival of *M. bovis* for less than eight weeks in the shade and less than four weeks in direct sunlight (Duffield and Young 1985). Tanner and Michel (1999), in a study in the Kruger National Park in South Africa, found that *M. bovis* survived for up to four weeks in spiked faeces and up to six weeks in buffalo lung or lymph node. Moisture and temperature were the major determinants of viability. Ultraviolet light was not thought to be a factor unless there was a lack of moisture, with maximum survival in faeces in sunny but moist conditions.

In New Zealand, Jackson *et al* (1995) looked at the survival of *M. bovis* adsorbed onto cotton strips and placed in different natural habitats. Maximum survival was found in possum dens (14-28 days in winter and spring, less than seven days in summer), then on the forest floor (14-28 days in winter and spring, less than four days in summer). No bacteria were found on pasture after four days.

Barron *et al* (2011) looked at survival times of *M. bovis* in possum carcasses in both grassland and forest in summer and winter in NZ. The average survival time for grassland was 3 days in summer and 20 days in winter. For forest habitat survival time was 9 days in summer and 27 days in winter.

The authors concluded that *M. bovis* does not survive long outside hosts, and that environmental contamination may therefore be relatively unimportant in the epidemiology of the disease (Jackson *et al* 1995).

M. bovis may be localised in the body, or it can be widely disseminated. Primary foci are usually in the respiratory and/or gastrointestinal tracts (Huchzermeyer *et al* 1994). Transmission of bovine tuberculosis is almost exclusively via the respiratory route, although infection can occur via alimentary, congenital, cutaneous, and venereal routes, as well as through the teat canal. *Mycobacterium bovis* is present in respiratory aerosols and may also be shed in vaginal secretions, milk, urine and faeces from cattle with generalised tuberculosis. Tuberculosis is usually spread to humans through unpasteurised milk and dairy products (Huchzermeyer *et al* 1994, Scanlon and Quinn 2000b).

Carcases and meat products:

Feeding of tuberculosis-infected carcasses poses some infective risk, particularly through infected lymph nodes (Huchzermeyer *et al* 1994). Meat harbours few or no tubercle bacilli, and the oral infective dose is large in comparison with the respiratory infective dose, leading Francis (1973) to conclude that the risk posed to humans by eating tuberculous meat was slight.

In Tanner and Michel's (1999) study in the Kruger National Park, *M. bovis* from naturally infected buffalo survived for up to six weeks in lung and lymph nodes in the winter and 5-14 days in the other seasons. The authors noted that previous investigations of *M. bovis* in possum and badger carcasses produced similar results, showing survival times of 2-4 weeks. Inactivation is aided by the decomposition of the carcass, and by the scavenging of vertebrates, insects and helminths.

Maximum survival for *M. bovis* buried in moist soil was five days. This was compared to the findings of O'Reilly and Daborn (1995), who were unable to isolate *M. bovis* from three buried badger carcasses after two, three, and six weeks respectively.

Merkal & Whipple (1980) found that inactivation of *M. bovis* in meat products by heat required time / temperature combinations 6-7°C below those formerly identified for the *M. avium* – *M. intracellulare* complex. A one log reduction required approximately 25 minutes / 53°C to one minute / 61°C. A five-log reduction required 350 minutes / 53°C to one minute / 68°C (all approximate figures read from graph). Ultraviolet radiation, amphy and formaldehyde vapour were found separately and in any combination to destroy *M. bovis* in thin meat emulsion smears.

Alkaline hydrolysis was found to completely inactivate *M. bovis* BCG in a study by Kaye *et al* (1998). This experiment involved the placement of 114-136 kg loads of animal carcasses into an animal digester, with pure cultures of various micro-organisms in dialysis bags also placed in the digester. The carcasses were covered by hot alkaline solution and kept at 110-120°C for 18 hours in the digester. The method was proposed as an alternative to incineration.

Milk and milk products: Large numbers of infective doses of *M. bovis* may be shed in the milk of infected cows (Huchzermeyer *et al* 1994). Drinking infected milk is a common method of spread in young animals (Radostits *et al* 2000).

Grant *et al* (1996) demonstrated inactivation of *M. bovis* in milk by heating at 63.5°C for 20 minutes, ten minutes within the specifications of holder or low-temperature, long time (LTLT) pasteurisation. Initial inocula were at a high level (10⁷ cfu/mL). *M. bovis* exhibited a linear thermal death curve and was second in heat sensitivity only to *M. fortuitum* of the five mycobacteria tested.

Harrington and Karlson (1965) have also shown that *M. bovis* does not survive LTLT or high-temperature, short time (HTST, 71.1°C /15 seconds) pasteurisation in skim milk. Huchzermeyer *et al* (1994), however, state that HTST pasteurisation is not always effective in destroying the bacilli, even when the time is extended to 20-25 seconds. The note of warning is a reminder that milk may contain a high number of cells or pus, which will reduce the efficacy of the pasteurisation in a way not observed by the *in vitro* studies.

If milk is inadequately pasteurised and made into sour milk, buttermilk, yoghurt and cream cheese, these milk products may contain *M. bovis* for up to 14 days after their preparation. In

butter the bacterium may survive for 100 days (Huchzermeyer *et al* 1994). The pH of sour milk does not destroy tubercle bacilli (Mattick and Hirsch 1946).

AQIS (1999a) gives examples of survival times for a range of cheeses:

- hard cheese 5-30 days
- semi-soft cheese 305 days
- camembert style soft cheese 47 days.

Keogh (1971) also reviewed survival times of *M. bovis* in a range of cheeses, and concluded that the requirement for minimum maturing periods in some countries (e.g. 60, 90 or 120 days before sale) could not be justified, because the survival time is so highly variable and can often exceed these periods. There was however a strong case for pasteurisation of all milk for cheese manufacturing.

Skins, hides and fibres: No specific reports were found on this aspect of the disease. *M. bovis* might be expected to found as a superficial contaminant of skins, hides and fibres, but its survival in the absence of moisture would be short. In a draft assessment, AFFA (2001) has concluded that these products do not pose a quarantine risk for bovine tuberculosis.

Semen/embryos: *M. bovis* may be present in the semen of infected bulls, originating from tuberculous lesions in the prepuce or when phagocytes containing *M. bovis* facilitate movement of the organism into the semen (Thoen *et al* 1977). *M. bovis* can survive in frozen semen. Venereal infection may occur by semen infected by both routes. The risk of transmission from infected bulls has been assessed as moderate (AQIS 1999b).

There is a reasonable likelihood that uterine flushes containing embryos from infected donor cows could contain *M. bovis*, as genital tuberculosis may occur in cows (AQIS 1999b). Rohde *et al* (1990) found that washing of embryos did not always remove other *Mycobacterium* spp. Some risk of transmission of *M. bovis* is therefore presumed despite washing of embryos.

Faeces: Scanlon and Quinn (2000a) describe numerous reports of *M. bovis* shed in the faeces of tuberculous and reactor cattle. There is an infective risk to grazing cattle if the slurry is spread on pasture (Jones 1980), although land used for tillage hay or silage is ideal for slurry from reactor herds (Scanlon and Quinn 2000b). Mechanical agitation of slurry can also create aerosols that are infective to animals or man (Scanlon and Quinn 2000a).

M. bovis that may survive for long periods in stored slurry and in the environment if spread on the land (Russell 1996). Scanlon and Quinn (2000b) found that *M. bovis* survived for six months in sterilised slurry in screw capped bottles, stored in the dark at an ambient temperature. Other reports include Williams and Hoy (1930) who reported survival for 4 months in slurry stored in a jar in an underground cellar, and Dokoupil (1964) who documented survival for 176 days in slurry at 5°C.

Scanlon and Quinn (2000a) describe two techniques of reducing the bacterial numbers to acceptable level. The first is long term storage, which is the slower but less expensive technique. It may be necessary to store the slurry for up to 6 months before all the *M. bovis* bacteria are inactivated naturally. The alternative is treat slurry with chemicals prior to spreading. Any chemical used must retain its activity in the presence of large amounts of organic matter.

The efficacy of five disinfectants against *M. bovis* were tested at varying concentrations in cattle slurry in screw-tapped bottles. Acetone (22.5%) was effective within 24 hours,

ammonium hydroxide (1%) within 36 hours, and chloroform (0.5%), ethyl alcohol (17.5%), and xylene (3%) within 48 hours (Scanlon and Quinn 2000a).

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Haemorrhagic septicaemia

Agent:

Pasteurella multocida

Agent type:

Bacterium

Persistence and inactivation:

General characteristics: Haemorrhagic septicaemia has been thoroughly reviewed by De Alwis (1999). Information on the survival of Pasteurellae under different conditions is scant (De Alwis, pers comm), probably because the existence of a carrier state in animals makes eradication by stamping out appear unfeasible. (Eradication has been attempted on Lombok, Indonesia, using mass vaccination. The disease does not appear to have been eradicated (De Alwis 1999).)

De Alwis (1999) states that “In general, *P. multocida* does not survive long enough outside the animal to become a significant source of infection, although survival may be longer in moist conditions”. The organism has been reported to survive for 2-3 weeks in sterilised soil (Bain *et al* 1982), although it could not be recovered from artificially infected, sterilised earth and mud from Malaysian rice fields after several hours exposure to sunlight (FAO 1959). Nor could it be recovered after 24 hours from mud in which buffaloes wallow (Bain *et al* 1982).

OIE (2016) state that *P. multocida* is susceptible to mild heat (55°C) and to most hospital disinfectants and that *P. multocida* can survive for hours and possibly days in damp soil or water but viable organisms are not found in the soil or pastures after 2–3 weeks.

Jung *et al* (2014) states that 3% hydrogen peroxide is an effective disinfectant for *P. multocida*

Carcases and meat products: No specific references to this aspect of the disease were found in the literature. It is believed that *P. multocida* can survive in animal tissues, including rotting carcasses, for a few days. Bacteria have been found at $10^{11} - 10^{12}$ CFU/mL of blood of carcasses 20-21 hours after death. Carcasses may be a source of infection during these few days, and the dumping of carcasses in rivers has been implicated in downstream spread of the disease (De Alwis 1999).

Milk and milk products: AQIS (1999a) have listed *P. multocida* (Asian strain, serotype B and African form serotype E) as an “organism not considered to be a quarantine hazard in dairy products”, noting that the agent is unlikely to be in the milk of animals producing milk for human consumption. No references were found during this review to the presence of *P. multocida* in milk.

Skins, hides and fibres: Skin is unlikely to be a source of infectivity, given that the organism spreads via ingestion of feed and water contaminated with nasal discharge and saliva, and does not survive more than few days in the environment. In an interim assessment, AFFA (2001) have classified *P. multocida* as not presenting a quarantine hazard in skins and hides.

Semen/embryos: There are no reports of *P. multocida* having been recovered from bovine semen, nor of haemorrhagic septicaemia being transmitted by artificial insemination in cows. However, other serotypes of *P. multocida* have been isolated from the prepuce and urine of healthy dogs, and these can be transferred from the dog to the bitch at mating. AQIS (1999b)

have therefore concluded that “it is likely that infected semen can transmit the infective organism to susceptible cows via artificial insemination” and permit importation of bovine semen only from HS-free countries. Similarly, there is a presumptive risk of *P. multocida* on embryos or in uterine flushes, and the same quarantine restriction is imposed.

Faeces: No references were found in the literature to the excretion of *P. multocida* in faeces.

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Glanders

Agent:

Burkholderia mallei

Agent type:

Bacterium

Persistence and inactivation:

General characteristics: *B. mallei* may be found in the urine, saliva, tears, faeces, nasal discharges and pus of infected animals (Bishop 1994). Transmission is mostly via ingestion from contaminated feed, water and utensils, and the cutaneous form of the disease (farcy) may arise from contamination of wounds. Transmission via inhalation has been demonstrated but its importance in the field is unknown (Radostits *et al* 2000). Khan *et al* (2013) states that house flies (*Musca domestica*) can play a role as a biological vector to transmit *B. mallei* from diseased to healthy animals (Lopez *et al.*, 2003). Vertical transmission from mare to foal and venereal transmission from stallions to mares have occurred.

B. mallei is relatively susceptible to environmental influences. Bishop (1994), citing other texts, notes that the bacterium is destroyed by sunlight in 24 hours, and by most common disinfectants, including phenol, formalin, chlorine, potassium permanganate and copper sulphate. *B. mallei* retains infectivity for three to five weeks in damp media, for 20-30 days in decomposing material, for about 20 days in clean water. Khan *et al* (2013) state survival of up to 100 days in water at room temperature and for about six weeks in contaminated stables. Shams *et al* (2011) inactivated the bacteria by applying a ClO₂ solution at a concentration of 0.25mg/L, at pH 7–8 and at 5-25°C.

OIE state that the bacterium is destroyed by heating to 55°C for 10 minutes or by UV irradiation (OIE, 2016). The bacterium is susceptible to many common disinfectants such as iodine, mercuric chloride in alcohol, potassium permanganate, benzalkonium chloride (1 part per 2000), sodium hypochlorite (500 ppm of available chlorine), 70% ethanol and 2% glutaraldehyde but is less susceptible to phenolic disinfectants.

Bleichert *et al* (2014) found that *B. mallei* was killed within 30 seconds of contact of copper surfaces. Candelieri *et al* (2016) found solutions of either 0.35% or 0.5% of stabilised peracetic acid used at temperatures between 23-30°C were effective against *B. mallei*.

Carcases and meat products: No specific references to this aspect of the disease were uncovered during this review. The appearance of glanders in lions in an Italian zoo has been attributed to the feeding of contaminated meat from imported horses (Battelli *et al* 1973). Bishop (1994) notes that, in South Africa, clinical cases of glanders must be destroyed and the carcasses burnt or buried. Khan *et al* (2013) states that deaths have been reported from carnivores including lions, tigers, leopards, domestic and feral cats, dogs, polar bears, wolves, jackals and hyenas after eating glanderous meat.

Skins, hides and fibres: AFFA (2001) has determined that there is some risk of *B. mallei* being found on the unprocessed skins of equids. As the agent is a non-spore forming bacillus, it is unlikely to survive the liming or acid pickling processes applied to skins.

Semen/embryos: No references were found on the presence of *B. mallei* in semen or on embryos.

Faeces: *B. mallei* may be found in the faeces of infected animals (Bishop 1994).

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Contagious equine metritis

Agent:

Taylorella equigenitalis

Agent type:

Bacterium

Persistence and inactivation:

General characteristics: Most, if not all, of the published information on the survival of *T. equigenitalis* relates to its survival for diagnostic purposes. Sahu *et al* (1979) compared the survival of the organism in exudate in the presence of three transport media, and in the absence of a transport medium. In day one, bacterial numbers decreased 15-fold at 22°C and two-fold at 4°C, but did not deteriorate at -70°C, in the absence of medium. Amies with charcoal was generally better than modified Amies without charcoal or Stuart's, with 19% of *Taylorella* surviving to day 10 at the three temperatures. Studies by Timoney *et al* (1979) showed the organism to be relatively heat labile, with thermal death times for pure culture in vaginal discharge ranging from at least 27 minutes at 40°C to less than one minute at 50°C. Sahu and Dardiri (1980) showed *T. equigenitalis* to be particularly susceptible to pH below 4.5.

Heath (pers comm) advised that “outside the body the organism would not be long-lived and in exudate alone at ambient temperature would most likely be dead within 10-14 days, maybe shorter – my opinion only. Chilling increases survival time but the role of a good transport medium is important to survival over time”. *T. equigenitalis* in dried exudates is susceptible to ten minutes of exposure to chlorhexidine diacetate (2%) or alkyldimethylbenzylammonium chloride (10%) (Henton 1994).

Allombert *et al* (2014) demonstrated the ability of the bacterium to survive in the environmental amoeba *Acanthamoeba castellanii* for up to one week.

Carcases and meat products: No studies on the survival of *T. equigenitalis* in carcasses were uncovered during this review. (Three independent experts reported a similar lack of information in the literature.) Heath (pers comm) speculated that faster growing commensal aerobes and anaerobes, as well as low pH, would inhibit the growth of *Taylorella*. Infection is limited to the reproductive tract of mares and surface contamination of the external genitalia of stallions (Timoney 1996).

Skins, hides and fibres: The risk of CEM infectivity via skins or hides should be negligible, given the normal route of transmission.

Semen/embryos: CEM can be transmitted by artificial insemination (Timoney 1996). The organism has frequently been isolated from the terminal urethra of carrier stallions, and less commonly from the pre-ejaculatory fluid of such animals. There is also the risk of indirect contamination of semen via materials and equipment carrying the organism. Timoney (pers comm) believes that provided high standards of breeding shed management are upheld, semen from non-infected stallions from CEM-infected premises can be used safely. However, decontamination of semen from carrier stallions using antimicrobials has not been successfully demonstrated (Timoney *et al* 1979).

Faeces: Any *T. equigenitalis* found in faeces would be an incidental contaminant. It is unlikely the organism would be found in significant numbers in faeces, or that it would pose any threat of infectivity, given the route of transmission.

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Potomac fever

Agent:

Ehrlichia risticii

Agent type:

Rickettsia

Persistence and inactivation:

Ehrlichia risticii is a rickettsia. It is an obligate intracellular parasite, seen principally in the bloodstream in association with monocytes. The agent is also found in various tissues and organs including small and large intestine and associated lymph nodes, and it is shed in faeces (Holland 1990, Radostits *et al* 2000).

The transmission of *E. risticii* has not been elucidated. It appears that the organism is not contagious. According to Palmer (1987), “it is difficult if not impossible to transmit using the faecal-oral route”, although Radostits *et al* (2000) cite reports of experimental transmission via the oral route, acknowledging that the significance of this in the natural disease is unknown. Infection can be artificially induced by injection of whole blood from an infected to a susceptible horse (Jenny 1984). The natural means of transmission is thought to take place via a vector, probably a tick. Transplacental infection has also been recorded (Holland 1990).

No reports were found on the survival of *E. risticii in vitro*, in carcasses or in animal products.

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Contagious bovine pleuropneumonia

Agent:

Mycoplasma mycoides subsp *mycoides* SC (bovine type)

Agent type:

Mycoplasma

Persistence and inactivation:

General characteristics: *M. mycoides* subsp *mycoides* SC is reported to survive off the host for up to three days in tropical climates and for up to two weeks in temperate zones (Schneider *et al* 1994). Laak (1992) states that pathology specimens may be stored at room temperature for weeks or months without affecting recovery of the agent. It survived for 216 hours in the shade, and 168 hours in the open on inoculated hay, and was inactivated by the combined effects of desiccation and UV light in a study by Windsor and Masiga (1977).

The organism is inactivated within 60 minutes at 50°C and within two minutes at 60°C, but may persist for at least twelve months in frozen lung tissue (Schneider *et al* 1994). OIE (2016) states inactivation within 60 minutes at 56°C and within 2 minutes at 60°C and up to 10 years in frozen tissue. It is killed below pH 5.5 (Windsor unpublished, cited in Windsor and Masiga 1977). The critical temperature for survival of the V5 vaccine strain that was used in Australia is 45°C (Hudson 1968).

M. mycoides subsp *mycoides* SC is sensitive to 1% phenol (three minutes to inactivation), 0.05% (OIE card states 0.5%) formaldehyde (30 seconds), and 0.01% mercuric chloride (one minute) (Provost *et al* 1987).

CBPP is spread by the inhalation of infected aerosols from the respiratory tract (Laak 1992) and there is a possibility of spread via infected droplets of urine (Schneider *et al* 1994). OIE (2016) states that the organism also occurs in saliva, urine, foetal membranes and uterine discharges and that transplacental infection can occur

Carcases and meat products: *M. mycoides* has been shown to survive in placenta for 72 hours, when it was overgrown by bacteria (Windsor and Masiga 1977). No specific references were found during this review regarding the survival of *Mycoplasma mycoides* in carcasses or meat. Transmission by ingestion of ground-up infected lung has been demonstrated experimentally (Hyslop 1959), although this study seems to have disappeared from most subsequent reviews.

Schneider *et al* (1994) state that “neither ingestion of infected fodder nor direct exposure of diseased organs of animals suffering from CBPP, will cause transmission”. AQIS (1999b) has determined that CBPP is unable to be transmitted by meat or meat products.

Milk and milk products: No references were found to the excretion of *M. mycoides* in milk. AQIS (1999a) has concluded that there is little risk of introducing the agent in milk. Even if the agent were present, experience with other *Mycoplasma* spp indicate that it should be inactivated by pasteurisation.

Skins, hides and fibres: Skins, hides and fibres are unlikely to harbour infective agent for long periods. In a draft assessment, AFFA (2001) has concluded that these products do not pose a quarantine risk for CBPP.

Semen/embryos: No reports were uncovered during this review of *Mycoplasma mycoides* appearing in semen or embryos. OIE (2016) state that microorganisms have also been isolated

from bull semen, but transmission through semen requires further investigation.

Faeces: *Mycoplasma mycoides* could be expected to be a contaminant of faeces. The agent is found in the urine of infected animals (Windsor and Masiga 1977).

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Windsor, R.S., & Masiga, W.N. 1977, 'Investigations into the epidemiology of contagious bovine pleuropneumonia: the persistence of *Mycoplasma mycoides* sub-species *mycoides* in placenta, urine and hay', *Bull An Health Prod Africa*, vol. 25, pp. 357-370.

Epizootic lymphangitis

Agent:

Histoplasma capsulatum var *farcimosum*

Agent type:

Fungus

Persistence and inactivation:

Little information of relevance is available for this agent.

Bardelli and Ademollo (1927) reported virulence of the organism after desiccation in the laboratory for 25 months. While Geering *et al* (1995) state that the organism persists for up to 15 days in the environment, Gabal and Hennager (1983) report much longer survival. Their study of five recently isolated strains showed that temperature and moisture content of soil affected viability. Maximum survival times in non-sterile soil and water for *H. capsulatum* were:

Temp (°C)	Soil (wks)	Water (wks)
-15	18	18
5	16	16
26	8	10
37	6	6

H. capsulatum is transmitted by direct contact, or via contamination of wounds by flies or fomites. There are also reports of transmission from stallions to mares during copulation (Al-Ani 1999).

Al-Ani (1999) recommends slaughter of infected animals in the case of exotic outbreaks. No references were found on the persistence of *H. capsulatum* in carcasses.

References:

Al-Ani, F.K. 1999, 'Epizootic lymphangitis in horses: a review of the literature', *Rev sci tech Off int Epiz*, vol 18, pp. 691-699.

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Geering, W.A., Forman, A.J., & Nunn, M.J. 1995, *Exotic diseases of animals: a field guide for Australian veterinarians*, Australian Government Publishing Service, Canberra.

Protozoa

Surra

Agent:

Trypanosoma evansi

Agent type:

Protozoan

Persistence and inactivation:

General characteristics: *Trypanosoma evansi* is a stercorarian trypanosome, i.e. there is no intermediate host in which the organism multiplies and undergoes morphological transformation. *T. evansi* is essentially transferred by mechanical means between mammalian hosts by biting insects, notably *Tabanus* spp (horseflies) and *Stomoxys* spp (stable flies) (Connor 1994).

According to Brun (pers comm) there are few or no reports on the *in vitro* or in carcase survival or inactivation of *T. evansi* in the literature. *T. evansi* is a fragile organism that disappears quickly in the environment or after the death of the host (Geering *et al* 1995, Brun pers comm). Several mainly foreign language papers on preservation for experimental purposes were uncovered during this review but were considered of little relevance.

Hashemi Fesharki (1981) reported that infected blood kept at -70°C for eight years killed all of five rats when inoculated at 1ml/rat.

Carcases and meat products: It is believed that *T. evansi* can be transmitted by ingestion of fresh carcasses. This has been confirmed in one case of a cat eaten by a canid, and the presence of infection in hyenas and other scavengers strongly suggests transmission by this route. The chance of human infection occurring via infected meat is considered negligible (Brun, pers comm).

The only objective study on survival in the carcase uncovered during this review was conducted by Sarmah (1998), who examined the viability (by microscopic examination and mouse inoculation) of *T. evansi* in the carcasses of parasitaemic rats stored at room temperature post mortem. All organisms were still viable at 4 hours; <1% were viable at 10 hours (although mice were infected); and by 12 hours all parasites had degenerated.

Mandal *et al* (2014) studied the survival and infectivity of *T. evansi* after the death of the host (mice, rats and rabbits). Results showed the presence of parasites 8 hours post-mortem without degeneration but marked degeneration of parasites was present from more than 10 hours after death of the host.

Brun (pers comm) agrees that once the host is dead, conditions are rapidly untenable for the parasite, and that the chance of survival in a carcase beyond 2-3 days is nil. The usual, 'fail-safe' process for disposing of experimental rodent carcasses infected with *T. evansi* at the Swiss Tropical Institute is to freeze/thaw and then incinerate them, although there is no empirical evidence for the efficacy of this approach.

Milk and milk products: *T. evansi* may be directly transmitted through milk (Wang 1988).

Skins, hides and fibres: Infectivity of trypanosomes via skins, hides or fibres is extremely unlikely given the fragile nature of the organism. In a draft assessment, AFFA (2001) has assessed these products as posing no quarantine hazard for the importation of trypanosomiases.

Semen/embryos: *Trypanosoma equiperdum* is found in the semen of horses (Brun *et al* 1998). Wang (1998) reported that *T. evansi* could be directly transmitted through coitus.

Faeces: No references were found to the presence of *T. evansi* in faeces.

References:

AFFA (Department of Agriculture Fisheries and Forestry – Australia) 2001, *Import risk analysis. Skins and hides. Draft report, August 2001*, AFFA, Canberra.

Brun, R., Hecker, H., & Lun, Z-R. 1998, '*Trypanosoma evansi* and *T. equiperdum*: distribution, biology, treatment, and phylogenetic relationship (a review)', *Vet Parasitol*, vol. 79, pp. 95-107.

Connor, R.J. 1994, 'African animal trypanosomiases', in *Infectious diseases of livestock, Vol I*, eds. Coetzer, J.A.W., Thomson, G.R., & Tustin, R.C., Oxford University Press, Cape Town, pp. 167-205.

Hashemi Fesharki, R. 1981, 'Survival of *Trypanosoma evansi*, *Theileria mutans*, *Babesia bigemina* and *Anaplasma marginale* following long-term maintenance at -70°C', *Arch Inst Razi*, vol. 32, pp. 47-50.

Mandal, M., Laha, R. & Sasmal, N.K., 2014. 'Experimental studies on survivality and degenerative changes of *Trypanosoma evansi* after death of host'. *Journal of Parasitic Diseases*, vol. 38(4), pp. 361-366.

Sarmah, P.C. (1998), 'Observation on survival of *Trypanosoma evansi* after death of the host', *J Vet Parasitol*, vol. 12(1), pp. 62-63.

Wang, Z-L. 1988, 'The similarities and differences of the characteristics between *T. equiperdum* and *T. evansi*', *Bul Vet Col (PLA)*, vol. 8, pp. 300-303 (in Chinese, cited in Brun *et al* 1998).

Dourine

Agent:

Trypanosoma equiperdum

Agent type:

Protozoan

Persistence and inactivation:

General characteristics: Like *T. evansi*, *Trypanosoma equiperdum* is a stercorarian trypanosome, having no intermediate host in which the organism multiplies and undergoes morphological transformation. However, *T. equiperdum* is unique among pathogenic trypanosomes in that it does not even require a vector for transmission between hosts. *T. equiperdum* has developed the capacity to survive in the genital tract and is spread directly by venereal means (Connor 1994).

Foals may also become infected, possibly at birth through infected vaginal discharges, udder lesions, or via infected milk (Schulz 1935).

According to Brun (pers comm) there are few or no reports on the *in vitro* or in carcase survival or inactivation of *T. equiperdum* in the literature. *T. equiperdum* is a fragile organism that disappears quickly in the environment or after the death of the host (Geering *et al* 1995, Brun pers comm). Several mainly foreign language papers on preservation for experimental purposes were uncovered during this review but were considered of little relevance.

Carcases and meat products: No references were found on the survival of *T. equiperdum* in carcasses or meat products. It seems reasonable to extrapolate from the study by Sarmah (1998) on survival of *T. evansi* in rat carcasses, in which no viable organisms were found after 12 hours. Brun (pers comm) agrees that once the host is dead, conditions are rapidly untenable for the parasite, and that the chance of survival in a carcase beyond 2-3 days is nil.

Milk and milk products: The infection of foals suggests that transmission by milk may be possible (Schulz 1935).

Skins, hides and fibres: Infectivity of trypanosomes via skins, hides or fibres is extremely unlikely given the fragile nature of the organism. In a draft assessment, AFFA (2001) has assessed these products as posing no quarantine hazard for the importation of trypanosomiasis.

Semen/embryos: *Trypanosoma equiperdum* is transmitted by coitus. The organism is found in the seminal fluid and mucous membranes of the genitalia of horses (Brun *et al* 1998).

Faeces: No references were found to the presence of *T. equiperdum* in faeces.

No further references were found in the 2017 review.

References:

AFFA (Department of Agriculture Fisheries and Forestry – Australia) 2001, *Import risk analysis. Skins and hides. Draft report, August 2001*, AFFA, Canberra.

Barrowman, P., Stoltz, W.H., van der Lugt, J.J., & Williamson, C.C. 1994, 'Dourine', in *Infectious diseases of livestock, Vol I*, eds. Coetzer, J.A.W., Thomson, G.R., & Tustin, R.C., Oxford University Press, Cape Town, pp. 206-212.

Brun, R., Hecker, H., & Lun, Z-R. 1998, 'Trypanosoma evansi and T. equiperdum: distribution, biology, treatment, and phylogenetic relationship (a review)', *Vet Parasitol*, vol. 79, pp. 95-107.

Connor, R.J. 1994, 'African animal trypanosomiases', in *Infectious diseases of livestock, Vol I*, eds. Coetzer, J.A.W., Thomson, G.R., & Tustin, R.C., Oxford University Press, Cape Town, pp. 167-205.

Sarmah, P.C. (1998), 'Observation on survival of *Trypanosoma evansi* after death of the host', *J Vet Parasitol*, vol. 12(1), pp. 62-63.

Schulz, K. 1935, 'Dourine or slapsiekte', *J Sth Afr Vet Med Ass*, vol. 6, pp. 4-15, cited in Barrowman *et al* 1994.

Agent:

Theileria parva parva

Agent type:

Protozoan

Persistence and inactivation:

The only natural means of transmission of *Theileria parva parva* is via the tick *Rhipicephalus appendiculatus*. Unlike the stercorarian trypanosomes such as *T. evansi*, however, the tick is critical in the life cycle, being the host for sexual reproductive phase of the organism.

Artificial transmission can be effected by injecting ground-up tick (or its salivary glands) containing sporozoites into the host. It can also be achieved by inoculation with suspensions of schizonts, from spleen, lymph nodes, blood, or culture, although this method is more effective when the cells originate from the donor (Lawrence *et al* 1994, Wilde 1967).

Destocking infected pastures of cattle for 15-18 months has eradicated infection, as this period exceeds the maximum lifespan of infected ticks (Lawrence *et al* 1994). Ochanda *et al* (2003) demonstrated the survival of *T. parva parva* in the nymphal ticks for up to one year. This highlights the role of the vectors as a reservoir of infection. These researchers also demonstrated that *T. parva parva* survived in the *R. appendiculatus* adult host for up to 78 weeks post salivary gland infection under quasi-natural conditions.

According to Morzaria (*pers comm*) there are few or no reports on the *in vitro* or in carcase survival or inactivation of *T. parva parva* in the literature, and none were uncovered during this review. *T. parva parva* is a fragile organism that disappears quickly in the environment or after the death of the host (Morzaria *pers comm*). Wilde (1967) noted that spleen used to induce experimental infection is less infective if taken immediately before or at death than if taken earlier. Several papers on preservation for experimental purposes were found but were considered of little relevance.

References:

Lawrence, J.A., de Vos, A.J., & Irvin, A.D. 1994, 'East Coast fever', in *Infectious diseases of livestock, Vol I*, eds. Coetzer, J.A.W., Thomson, G.R., & Tustin, R.C., Oxford University Press, Cape Town, pp. 309-325.

Ochanda, H., Young, A.S. and Medley, G.F., 2003. Survival of *Theileria parva* in its nymphal tick vector, *Rhipicephalus appendiculatus*, under laboratory and quasi natural conditions. *Parasitology*, vol 126(06), pp.571-576.

Ochanda, H. and Young, A.S., 2003. Survival of *Theileria parva*-infected adult *Rhipicephalus appendiculatus* under laboratory and quasi-natural conditions. *Experimental & applied acarology*, 29(3-4), pp.355-365.

Wilde, J.K.H. 1967, 'East Coast fever', in *Advances in Veterinary Science Volume II*, eds Brandly, C.A., & Cornelius, C., Academic Press, New York, pp. 207-259.

Equine babesiosis (piroplasmosis)

Agent:

Babesia equi and *Babesia caballi*

Agent type:

Protozoa

Persistence and inactivation:

As with *Theileria parva parva*, an intermediate tick host is critical to the life cycles of *Babesia equi* and *B. caballi*. Several ticks are implicated in transmission (*Rhipicephalus* spp, *Hyalomma* spp, and *Dermacentor* spp). Disease has been induced by inoculation of infected blood into susceptible animals, and iatrogenically via contaminated needles (de Waal and van Heerden 1994).

No reports of the *in vitro* or in carcase survival or inactivation of *B. equi* or *B. caballi* were found in the literature. As with other protozoa, preservation of live organisms is difficult. *Babesia bovis* can be detected in heart, lung and kidney up to eight hours after death, and up to 28 hours in brain (Radostits *et al* 2000). Several papers on preservation for experimental purposes were found but were considered of little relevance.

No further references were found in the 2017 review.

References:

De Waal, D.T., & van Heerden, J. 1994, 'Equine babesiosis', in *Infectious diseases of livestock, Vol I*, eds. Coetzer, J.A.W., Thomson, G.R., & Tustin, R.C., Oxford University Press, Cape Town, pp. 295-304.

Radostits, O.M., Gay, C.G., Blood, D.C., & Hinchcliff, K.W. (eds) 2000, *Veterinary medicine*, ninth edition, W. B. Saunders Company Ltd, London.

Multicellular parasites

Sheep scab

Agent:

Psoroptes ovis

Agent type:

Mite

Persistence and inactivation:

Psoroptes ovis is an obligate parasite. Its survival off the host has been the subject of widely differing estimation and opinion (summarised in O'Brien *et al* 1994). The study coming closest to mimicking natural conditions appears to be that of O'Brien *et al* (1994), in which the mites retained infectivity for up to 16 days under ambient conditions in Ireland. Survival and infectivity was consistent across all seasons and in the refrigerator. Some mite eggs hatched after a week off the host when subsequently placed in an incubator. The eggs hatched in 1-3 days after placement in the incubator or not at all.

Using artificial environments, other workers have reported longer survival times (for example, 48 days in the laboratory for a bovine strain by Liebisch *et al* 1985). Smith *et al* (1999) showed that temperature and humidity affected survival of *P. ovis*. Maximum survival times of *P. ovis*, maintained in chambers and supplied with distilled water, were 7-8 days at 24-26°C, and 15-18 days at 2-9°C. The closely related species *P. caniculi* from rabbits appeared to have significantly lower survival below a relative humidity of 65-75% at 30°C.

Meintjes *et al* (2002) looked at off-host survival times of *P. ovis* at 10°C. The recorded survival was between 9.25 and 15 days depending on the life stage of the mite. Eggs were shown to hatch for up to 31 days post removal from the host. This study recommended leaving enclosures in which infected sheep had been housed empty for at least 17 days (but up to 30 in winter).

P. ovis is susceptible to several ectoparasiticides, and is usually treated by topical treatment using an organophosphate (e.g. diazinon 0.01%, propetamphos 0.0125%). Ivermectin may also be effective (Radostits *et al* 2000).

P. ovis is an external parasite and therefore not associated with meat, milk, semen/embryos or faeces. Survival of the mite on carcasses might be expected to fall within the range of estimates described above, but no specific studies on survival on carcasses were found during this review (the lack of data was confirmed by Wall, pers comm).

In a draft assessment, AFFA (2001) has concluded that the risk of spreading *P. ovis* by raw hides or wool is slight. *P. ovis* is unlikely to survive the drying process of hides and scouring of wool.

References:

AFFA (Department of Agriculture Fisheries and Forestry – Australia) 2001, *Import risk analysis. Skins and hides. Draft report, August 2001*, AFFA, Canberra.

Liebisch, A., Olbrich, S., & Deppe, M. 1985, 'Survival of *P. ovis*, *P. cuniculi*, *C. bovis* when separated from the host animal', *Dtsch Tieraerztl Wochenschr*, vol. 92, pp. 165-204, cited in O'Brien *et al* 1994 and Smith *et al* 1999.

Meintjes, T., Fourie, L.J. & Horak, I.G., 2002. 'On-host ecology and off-host survival of the sheep scab mite *Psoroptes ovis*', *The Onderstepoort journal of veterinary research*, vol. 69(4), pp. 273-283.

O'Brien, D.J., Gray, J.S., & O'Reilly, P.F. 1994, 'Survival and retention of infectivity of the mite *Psoroptes ovis* off the host', *Vet Res Comm*, vol. 18, pp. 27-36.

Radostits, O.M., Gay, C.G., Blood, D.C., & Hinchcliff, K.W. (eds) 2000, *Veterinary medicine*, ninth edition, W. B. Saunders Company Ltd, London.

Smith, K.E., Wall, R., Berriatua, E., & French, N.P. 1999, 'The effects of temperature and humidity on the off-host survival of *Psoroptes ovis* and *Psoroptes cuniculi*', *Vet Parasitol*, vol. 83, pp. 265-275.

Screw-worm fly

Agent:

Chrysomya bezziana (Old world screw-worm fly), *Cochliomyia hominivorax* (New world screw-worm fly)

Agent type:

Fly

Persistence and inactivation:

The screw-worm fly (SWF) lay eggs on the dry edge of wounds or body orifices. Eggs hatch within 12-20 hours, and the first instar larvae burrow into the wound and begin to feed. Moults to second and third instar larval stages occur after successive periods of 24 hours. The larvae feed until 6-7 days old then drop off to pupate in the soil. Pupation lasts for a week to two months, depending on ambient conditions. Adult males and female flies emerge and commence mating, with females laying the first batch of eggs after 6-7 days. The life cycle can be completed in 20 days in optimal conditions. The average adult lifespan of the SWF is 21 days (Spradbery 1994, Geering *et al* 1995).

Screw-worm flies will probably not survive in open areas subject to dry heat, unless vegetation is available for shade and carbohydrate. SWF prefers an ambient temperature of 20-30°C, will not move at <10°C and may not mate at 10-16°C. It does not survive in areas that experience frosts (Geering *et al* 1995).

The OIE state that pupae are destroyed in soil at temperatures <8°C and that effective chemicals/disinfectants are: organophosphate insecticides; carbamate and pyrethroid compounds (OIE 2016). The preferred prophylactic treatment for livestock in the face of a screw-worm fly incursion is ivermectin. A recent study showed that ivermectin boluses protected cattle from 14 to 21 days after treatment and inhibited fly breeding in dung, although with negative impacts on dung beetles (Wardaugh *et al* 2001).

The following recommendation for the treatment of screw-worm fly are made (OIE 2016): Organophosphate insecticides (e.g. coumaphos, dichlofenthion, fenclorphos) have been used for the treatment of wounds infested with old world screw-worm fly (OWS) and new world screw-worm fly (NWS) larvae resulting in larvae being expelled and dying on the ground. Reapplication of insecticides at 2-3 day intervals until infected wounds have healed is recommended to prevent reinfestation (OIE 2016).

Treatment using 5 grams of 5% coumaphos wettable powder either sprinkled directly onto a wound or, more effectively, brushed into the wound as a paste after mixing with ordinary cooking oil (33 ml) is recommended.

Organophosphorus compounds may also be applied as aerosol sprays or as dusts that are puffed into the wound using plastic squeeze bottles

Direct prevention of screwworm infestation can be achieved by spraying or dipping of livestock with coumaphos (0.25% aqueous suspension of 50% wettable powder) or other organo-phosphorus insecticides at the maximum concentration prescribed for external parasite control

There are few recent studies that assess efficacy of insecticides for screwworm treatment and control, but many older publications describe the benefits of various macrocyclic lactones – especially subcutaneous injections of doramectin – in preventing infestation of umbilical or

castration wounds of calves and of infestation in post-parturient cows for up to 12–14 days post-treatment

Topical application of 10 mg/kg bodyweight of a 1% fipronil solution did not prevent oviposition by NWS, but reduced the proportion of bulls developing active myiasis over the critical 10-day post-castration period. Similarly, topical application of an insect growth regulator (IGR), dicyclanil, to castration wounds in cattle gave good protection (>90%) against NWS myiasis

Larval screw-worm flies are unlikely to persist long on carcasses or skins, as they rely on blood for nourishment. AUSVETPLAN (Animal Health Australia 2007) state that there is some quarantine risk posed by unprocessed hides from SWF-endemic countries. It noted that treatment with methyl bromide, ethylene oxide or other insecticidal gases would be effective with individual hides but was unlikely to penetrate layers of hides, so post-border processing in quarantine would be necessary.

AUSVETPLAN (Animal Health Australia 2007) also notes that the only animal product necessary to treat is faeces, as it may contain pupating larvae. The preferred product is bromophos plus chlorfenvinphos, with phosmet, crotoxyphos or cypermethrin plus chlorfenvinphos as alternatives.

Eradication of the disease (New World Screw-worm Fly) may be possible through the Sterile Insect Technique (SIT) Gutierrez et al (2004)

References:

AFFA (Department of Agriculture Fisheries and Forestry – Australia) 2001, *Import risk analysis. Skins and hides. Draft report, August 2001*, AFFA, Canberra.

Animal Health Australia, 2011, 'Disease strategy: Screw-worm fly (Version 3.0)' In: *Australian Veterinary Emergency Plan (AUSVETPLAN), Edition 3*, Primary Industries Ministerial Council, Canberra, ACT.

Geering, W.A., Forman, A.J., & Nunn, M.J. 1995, *Exotic diseases of animals: a field guide for Australian veterinarians*, Australian Government Publishing Service, Canberra.

Gutierrez, A.P. and Ponti, L., 2014. The new world screwworm: prospective distribution and role of weather in eradication. *Agricultural and Forest Entomology*, 16(2), pp.158-173.

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Spradbery, J.P. 1994, 'Screw-worm fly: a tale of two species', in *Agricultural Zoology Reviews*, vol. 6, October 1994, ed. Evans, K., Intercept Ltd, Andover, pp. 1-61.

Wardaugh, K.G., Mahon, R.J., & Ahmad, H.B. 2001, 'Efficacy of macrocyclic lactones for the control of larvae of the Old World Screw-worm Fly (*Chrysomya bezziana*)', *Aust vet J*, vol. 79, pp. 120-124.

Agent:

Trichinella spiralis

Agent type:

Nematode

Persistence and inactivation:

General characteristics: *T. spiralis* is a nematode. Adults are present in the intestine. Fertilised females burrow into the villi and produce L1 larvae, which enter the lymphatics and travel via the bloodstream to the skeletal muscles. They penetrate muscle cells and become encapsulated by the host. The larvae resume development when the muscle tissue is eaten by another host. Larvae remain infective in living hosts for many years. Transmission also occurs by ingestion of fresh faeces from animals with a patent infection.

Riva et al (2012) looked at the survival of *T. spiralis* larvae in mouse carcasses in summer and winter in Argentina. Results demonstrated survival of *T. spiralis* larvae and infectivity of muscle for a 1-week period in summer and for at least for 6 weeks in winter.

Trichinella have a wide range of hosts, including some aquatic mammals. Crustaceans and fish may have a role as transport hosts in these infections (Urquhart *et al* 1987).

Carcases and meat products: *Trichinella* is spread by feeding of swill containing under-cooked pig flesh to other pigs. Rodents and other rodents also act as a reservoir of infection.

Oivanen et al looked at the persistence of *T. spiralis* in feed sources contaminated by infected rodents. *T. spiralis* infected rat carcasses were incubated for 6 weeks in several animal feeds to assess how long the contaminated feed presented risk of an outbreak. Infected target rats were placed into silage, grain barley, propionic acid-preserved feed, and simulated pasture. A two-week incubation period resulted in reduced numbers of infective larvae in all feedstuffs. Low numbers were isolated from all propionic acid fermented-feeds after four weeks. This supports the possibility of farm animals becoming infected from eating hay or other feeds that were contaminated by infected rats. Silage should ideally be stored for at least one month before use to negate any (small) risk from this forage.

Based on the discovery in Serbia in 2002 of a *T. spiralis* infected horse, Murrell et al (2004) determined that 32% of 219 horses would eat meat patties. Three horses were offered pork balls containing *T. spiralis* larvae and subsequently became infected. They concluded that horses are more willing to consume meat than originally realised. The feeding of animal products and kitchenwaste is a common occurrence among horse owners in some countries. Limiting horse access to meat-contaminated feedstuffs should be managed in the event of an outbreak.

According to Urquhart *et al* (1987), *Trichinella* maintain infectivity in decomposing carcasses for several months. Jovic *et al* (2000) studied the survival of *T. spiralis* larvae in 700g pieces of pig muscle buried at depths of 30, 50 and 100cm at a temperature between 4-13°C. Infectivity was maintained throughout the 91-day experimental period at all depths. Typical putrefaction with marked odour and tissue decay was not observed, reflecting the low temperatures in the soil. The authors compared their results with those of Modic (1976), who reported survival of larvae between 25 and 40 days at 30°C. Hill et al (2007) showed that infective *T. spiralis* – a non-freezing tolerant species – can survive for at least 4 weeks in

horse tissue frozen at either -5°C or -18 °C but that the number of infective larvae decreases substantially by day 2 at -18°C and by week 4 at -5°C.

The International Commission on Trichinellosis has published standards for the control of *Trichinella* in pork for human consumption (Gamble *et al* 2000). The ICT states that there are three acceptable methods for inactivating *Trichinella spiralis* in meat: cooking, freezing, and irradiation.

For cooking, the ICT recommends heating according to one of a series of time and temperature combinations set out by the United States Department of Agriculture's Code of Federal Regulations (USDA, 1990) (see table).

Min. int. temp. (°C)	Min. time
49.0	21h
50.0	9.5h
51.1	4.5h
52.2	2h
53.4	1h
54.5	30min
55.6	15min
56.7	6min
57.8	3min
58.9	2min
60.0	1min
61.1	1min
62.2	instant

The standards further stipulate that the time to raise the product from 15.6°C to 49.0°C should not exceed 2h unless the product is cured or fermented, and that steps be taken to ensure all parts of the product are heated through. In the absence of proper temperature and time control and monitoring systems, the meat should be checked to ensure colour has changed from pink to grey throughout, and the texture such that muscle fibres are easily separated.

Randazzo *et al* (2010) found that cooking for 15 minutes at 90°C was needed to kill 100% of free larvae and for 15 minutes at 100°C to kill 100% of encysted larvae. These temperature and time combinations are significantly longer than the USDA recommendations. The authors suggest that potentially inefficient temperature and exposure time charts have been used to control *T. spiralis*.

Smoking, drying or curing pork does not necessarily inactivate *Trichinella* larvae (Urquhart *et al* 1987). However, Medina-Lerena *et al* (2009) looked at different processing procedures on the reproductive capacity of *T. spiralis* in pork meat. Along with freezing they found that drying for 24 hours at 60°C with subsequent cold storage was effective at eliminating the muscle larvae infectivity. The process involved first drying the meat, then bagging and storage at a constant temperature of 4°C for 15, 45, 60, 75, 90, 105 or 266 days after preparation.

For freezing, the ICT again recommends guidelines from the USDA's Code of Federal Regulations (USDA 1990). The Code specifies the following minimum times at various maximum temperatures:

Max. internal temp. (°C)	Group 1* (days)	Group 2** (days)
-15	20	30
-23	10	20
-29	6	12

* pieces not greater than 15cm thickness

** pieces between 15cm and 69cm thickness

Where proper time and temperature control and monitoring systems are not available, pieces of meat up to 15cm thick should be frozen solid (>15°C) for at least three weeks, and for meat 15-69cm thick, for at least four weeks. These recommendations apply to pig and horse meat, but not game meats, as these often harbour freeze-resistant species of *Trichinella*. (Pigs in certain regions may also be infected with freeze-resistant *Trichinella*.)

A broader range of time/temperature options for freezing is provided by Kotula *et al* (1990), who studied the inactivation of *T. spiralis* in pork at temperatures ranging from -193°C to -1°C, for periods from 1 second to 182 days. Thermal death curves included the following points:

Temp °C	Predicted thermal death time	Predicted 99% confidence limit	Predicted 99.999% confidence limit
-10	4.0 days	266 days	NR
-15	64 min	63 hrs	180 days
-20	8 min	48 min	21 days

NR not reported

A study by Lacour *et al* (2013) showed that freezing at -21°C for 1 week inactivated *T. spiralis* muscle larvae encapsulated in wild boar meat by 24 weeks.

Porto-Fett *et al* (2010) demonstrated that fermentation and drying and/or High Pressure Processing (483 and 600 MPa for 0.5 to 5 min.) of contaminated Genoa salami or pork are effective for inactivating *T. spiralis* larvae. High pressure processing may be used as an alternate to curing for trichinae control.

For irradiation, the ICT recommends 0.3 kilogray (for use on sealed packaged food only).

Milk and milk products: No reports were found of *Trichinella* larvae in milk or milk products.

Skins, hides and fibres: These products are highly unlikely to pose an infective risk.

Semen/embryos: No reports were found of *Trichinella* larvae in semen or on embryos.

Faeces: Larvae may be found in faeces of patent, infected animals (Urquhart *et al* 1987). No references were found regarding persistence of larvae in faeces.

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Appendix 1

Example search strategy – 2001 report

DATABASES SEARCHED

Database: Medline

Version: PubMed (<http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=PubMed>)

Files searched: all

Date searched: 14-Sep-01

Search strategy:

babesiosis [MESH] AND horses [MESH]

equine babesiosis OR equine piroplasmosis

1 OR 2

survival OR persist* OR inactivat* OR disinfect*

3 AND 4

review OR review literature OR review of reported cases OR review, academic OR review, multicase OR review, tutorial OR scientific integrity review [PUBLICATION TYPE]

3 AND 6

5 OR 7

Database: CAB Abstracts

Version: SilverPlatter on WebSpirs

Files searched: 1972-2001/07

Date searched: 14-Sep-01

Search strategy:

equine babesiosis OR equine piroplasmosis

babesia equi OR babesia caballi

2 OR 3

(virus OR agent OR disease) NEAR (survival OR persist* OR destruct*)

(viral OR virus OR agent)NEAR (inactivat* OR disinfect* OR persist*)

4 OR 5

3 AND 6

review in ti,ab

3 AND 8

7 OR 9

Database: Agricola

Version: SilverPlatter on WebSpirs

Files searched: 1992-2001/06

Date searched: 14-Sep-01

Search strategy: As above for CAB Abstracts

Database: ABOA

Version: SilverPlatter on WebSpirs

Files searched: 1975-

Date searched: 14-Sep-01

Search strategy: As above for CAB Abstracts

Database: Current Contents

Version: OVID at <http://gateway.ovid.com/>

Files searched: AGR, LIFE Week 29 2000 - week 38 2001
Date searched: 14-Sep-01
Search strategy: As above for CAB Abstracts

Example search strategy – 2017 review

DATABASES SEARCHED

Database: Web of Science

Files searched: All Databases

Search Period: Aug 2016-Feb 2017

Search strategy: (using rinderpest as an example) was as follows:

- #1: TS=(rinderpest OR RPV OR 'cattle plague' OR 'steppe murrain')
- #2: #1 with publication date > 2000
- #3: TS=(survival OR persist* OR inactiv* OR disinfect*)
- #4: #2 AND #3
- #5: TS=('review') [If more than 50 papers identified at step 4]
- #6: #4 AND #5

[‘#’ is the identifier of each individual search. Individual searches can be combined using terms such as AND , OR, NEAR, etc.]