Persistence of Disease Agents in Carcases and Animal Products

Report for Animal Health Australia

by

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Table of Contents

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 animal disposal methods in Australia and to augment the Disposal manual of AUSVETPLAN.

Terms of reference for the 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 carcases 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:*
> - *Left in situ, i.e. no further treatment*
> - *Burial*
> - *Above-ground 'burial'*
> - *Aerobic decomposition*
> - *Burial at sea*
> - *Chilling*
> - *Rendering*
> - *Combustion.*

Methodology

The 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. This update, with modification to the conclusions, was completd in December 2003.

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, and 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 carcases and animal products. In particular, there 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.

   Reasons for this lack of information include low relevance to the epidemiology or control of 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.

3. For each of the disease agents in the report, the following information is presented:
   - General characteristics of the agent and of the disease that are relevant to transmission via carcases 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
   - 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
   - Conclusions based on the available data for disposal of carcases and products
   - References and expert contacts made.

4. This revised version of the report departs from the letter of the original terms of reference. The paucity of literature, for virtually all of the agents studied, precluded firm conclusions being drawn about persistence following disposal by each of the methods presented. The 'Conclusions' section for each agent has been revised, so that it now summarises key points and makes recommendations based only on known information and reasonable extrapolation.**These recommendations should not necessarily be considered as policy statements by Animal Health Australia, the Australian or State/Territory governments.**

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^{o}C and 1.5 hours at 37^{o}C. Stability is greater in citrated whole blood, where the presence of protein is protective: 66 days at 5^{o}C, 33 days at 15-18^{o}C, 42 hours at 37^{o}C, 1.5 hours at 50^{o}C, and 5 minutes at 60^{o}C. NSDV is sensitive to lipid solvents and detergents and is rapidly inactivated outside the optimum pH range (Terpstra 1990, Montgomery 1917).

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 carcases or muscle during the conduct of this review. The virus would be expected to disappear quickly from the carcase 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 carcases.

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.

Conclusions:

- **Nairobi sheep disease virus is a fragile virus outside its narrow optimal pH range. It is inactivated by the pH changes associated with rigor mortis in meat.**
- **NSDV is spread by a tick vector.**
- **Carcases and animal products present a negligible disease risk and no special precautions for disposal are warranted.**

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.

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. It is also inactivated by lipid solvents, including ether and sodium deoxycholate, and low concentrations of formalin (Swanepoel and Coetzer 1994).

RVF is transmitted by a number of mosquitoes, 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 foetuses 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.

Conclusions:

- **Rift Valley fever virus can readily survive in body fluids between pH7-9, but is fragile outside this pH range. It is inactivated by the pH changes associated with rigor mortis in meat.**
- **RVF is principally spread by mosquitoes, but human infection can also occur by contacting fresh tissues or body fluids from an infected animal.**
- **Burial, burning and rendering are the preferred methods for disposal of fresh carcases.**
- **Meat, skins, hides and fibres present a negligible transmission risk.**
- **Milk should be pasteurised to inactivate the RVF virus.**
- **Semen and embryos should be destroyed.**

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.

DPIE (Department of Primary Industries and Energy) 1996, *Australian Veterinary Emergency Plan, AUSVETPLAN Edition 2.0, Disease Strategy Rift Valley Fever,* DPIE, Canberra.

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.

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.

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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).

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 carcases 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.

Conclusions:

- **Japanese encephalitis virus is a fragile virus which can only survive for a few hours away from an animal host.**
- **JEV is spread by a mosquito vector.**
- **Carcases and animal products present a negligible transmission risk and no special precautions for disposal are warranted.**
- **Semen and milk from infected or suspect animals should not be used.**

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.

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^{o}C for 30 minutes but are stable at temperatures below -60^{o}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 carcase 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 carcases 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 carcases 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 (Jezyna 1976). The virus is totally inactivated by pasteurisation using "normal" pasteurisation temperature / time treatments (Reid pers comm).

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 [louping-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.

Conclusions:

- **Tickborne encephalitis complex viruses are sensitive agents which are unlikely to survive for more than a few days in animal products in the field.**
- **TBEC viruses are normally spread by a tick vector, but transmission via ingestion cannot be ruled out.**
- **Carcases and animal products present a low transmission risk but should be disposed of using a method that prevents scavenging during the first week.**
- **Milk should only be used following pasteurisation.**

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, *An analysis of the disease risks, other than scrapie, associated with the importation of ovine and caprine semen and embryos from Canada, the United States of America and member states of the European Union, final report,* AQIS, Canberra.

Bannatyne, C.C, Wilson, R.L., Reid, H.W., Buxton, D., & Pow, I. 1980, 'Louping-ill virus infection of pigs', V*et Rec*, vol. 106, p. 13.

Gresikova, M., & Kaluzova, M. 1997, 'Biology of tick-borne encephalitis virus', *Acta virologica,* vol. 41, pp. 115-124.

Gresikova, M., Sekeyova, M., Stupalovaa, S., & Necas, S. 1975, 'Sheep milk borne epidemic of tick-borne encephalitis in Slovakia', *Intervirology,* vol. 5, p. 57, cited in Gresikova and Kaluzova 1997.

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Reid, H.W., Buxton, D., Pow, I., & Finlayson, J. 1984, 'Transmission of louping-ill virus in goat milk', *Vet Rec,* vol. 114, pp. 163-165.

Swanepoel, R. 1994, 'Louping ill', in *Infectious diseases of livestock, Vol II,* eds. Coetzer, J.A.W., Thomson, G.R., & Tustin, R.C., Oxford University Press, Cape Town, pp. 671-677.

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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 sensitive to acidity (< pH 8.0), temperatures above 40^{o}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 carcases has caused infection in humans, and aerosol transmission may be possible, but it appears there is no direct spread between sheep (Swanepoel and Coetzer 1994).

Carcases 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 Wesselsbron disease virus as an agent that may be excreted in milk, but which is not considered to pose 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.

Conclusions:

- **Wesselsbron disease virus is a sensitive agent which is unlikely to survive for more than a few days in carcases or animal products in the field.**
- **Wesselsbron disease is principally spread by mosquitoes, but human infection can also occur by contacting fresh tissues or body fluids from an infected animal.**
- **Carcases and animal products present a low transmission risk but should be disposed of using a method that prevents scavenging.**

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) 1999, *Import risk analysis: Importation of dairy products for human consumption,* AQIS, Canberra.

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Orthomyxiviridae

Avian influenza (highly pathogenic)

Agent:
Family Orthomyxiviridae, type A

Agent type:
Virus

Persistence and inactivation:
Highly pathogenic avian influenza 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), while 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. HPAIV is stable between pH 5.5 and 8 (DPIE 1996). 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. Storage of the virus at -70°C or lyophilisation is recommended for long-term maintenance of infectivity (Easterday *et al* 1997).

HPAIV is destroyed by lipid solvents such as detergents, as well as formalin, beta-propiolactone, oxidising agents, dilute acids, ether, sodium desoxycholate, hydroxylamine, sodium dodecylsulphate, and ammonium ions (Easterday *et al* 1997).

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).

Carcases and meat products: Swayne and Suarez (2000) note, with regard to 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.

DPIE (1996) states that the virus survives several days in carcases 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 carcases. The composting was performed in bins at an ambient temperature of 22°C. Various organs of experimentally-infected birds were composted with poultry carcases 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: 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.

A number of 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 more hardy) 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 10^7 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 presence of organic material protects the virus. Manure can be disposed of by burial, composting in a pile covered by plastic, or rototilling[1] (Easterday *et al* 1997).

Conclusions:

- **Highly pathogenic avian influenza virus can survive in organic matter and water for months. However, the virus is quite sensitive to heat and is inactivated by a wide range of disinfectants.**
- **The HPAI virus is highly contagious. Biosecurity of potentially infective material is most important.**
- **Burning and burial are the preferred methods for disposal of infective material.**

[1] A definition for this word could not be found

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Equine influenza

Agent:
Family Orthomyxiviridae, 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^{o}C for 30 minutes. It was quickly inactivated by savlon, dettol, phenyl, alcohol, formalin, and potassium permanganate but not by sodium carbonate (AUSVETPLAN (DPIE 1996) advocates 4% lysol). EIV persisted for up to 14 days in tap water at 4^{o}C, or 2 days at 37^{o}C, and in canal water (where colloids were thought to be protective) for 1-2 weeks at 22^{o}C or 37^{o}C. It survived in urine at pH 8.0 for up to 5 days and in soil at 18^{o}C for one day.

EIV is spread via the respiratory route, almost exclusively by direct contact between horses. Indirect spread by people and fomites may play some role (Mumford 1994).

Carcases and meat products: No reports on the persistence of equine influenza in horse carcases were uncovered during the course of 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).

Conclusions:

- **Equine influenza virus is a sensitive virus which is unlikely to survive for more than a few days away from an animal host. It is readily inactivated by exposure to UV light.**
- **EIV is spread by the respiratory route, almost exclusively with close contact between horses.**
- **EIV may be present in semen and embryos from infected horses, but transmission is unlikely to occur by this route.**
- **Carcases and animal products present a negligible transmission risk and no special precautions in disposal are warranted.**

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Swine influenza

Agent:
Family Orthomyxiviridae, type A

Agent type:
Virus

Persistence and inactivation:
General characteristics: Swine influenza virus is very labile, being rapidly inactivated by all detergents and disinfectants. 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^{o}C for between 21 and 28 days, and also 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 were able to isolate virus for between 8 and 15 days at -20^{o}C. Loss of infectivity was slightly slower at 4^{o}C compared to -20^{o}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 (1990) found that time to inactivation for SIV in slurry varied between 9 weeks at 5^{o}C to one hour at 50^{o}C (detection limit 0.7 $\log_{10}$ $TCID_{50}$/50L, initial dose $10^{5.8}$ $TCID_{50}$/50L).

Conclusions:

- **Swine influenza virus is a sensitive virus which is unlikely to survive for more than a few days away from an animal host. It is readily inactivated by heat and exposure to UV light.**
- **SIV is spread by the respiratory route, with close contact required between infected and susceptible pigs.**
- **Carcases and animal products present a negligible transmission risk. The only special precaution is that the method disposal must preclude scavenging by feral pigs.**

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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).

Conclusions:

- **There is insufficient information available to make detailed recommendations on the disposal or disinfection of animal carcases or products exposed to Hendra virus.**

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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 CTC (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 considered to be 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).

Conclusions:

- **There is insufficient information available to make detailed recommendations on the disposal or disinfection of animal carcases or products exposed to Menangle virus.**

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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).

NDV is sensitive to a wide range of disinfectants, including chlorine-based chemicals and lipid solvents (AFFA 2000).

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).

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 carcases 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 carcases held at 2°C
- 160 days on the skin and 196 days in bone marrow of eviscerated unplucked carcases held at 2°C
- greater than 300 days on skin and in bone marrow of both plucked and unplucked eviscerated carcases 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^{o}C is suggested as sufficient for meat with low titre NDV. For waste foods fed to poultry, one minute at 100^{o}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^{o}C, 10^{o}C, 20^{o}C, and 30^{o}C. pH dropped as low as 3.5 at 30^{o}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 also 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^{o}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).

Conclusions:

- **Newcastle disease virus can survive in organic matter for months. However, the virus is quite sensitive to heat and is inactivated by a wide range of disinfectants.**
- **The ND virus is highly contagious. Biosecurity of potentially infective material is very important.**
- **Burning, rendering and burial are the preferred methods for disposal of infective material.**

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Nipah virus

Agent:
Family Paramyxoviridae, genus megamyxovirus (proposed name)

Agent type:
Virus

Persistence and inactivation:
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 also 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).

Little information is available on the survival and inactivation properties of this virus (Daniels, Kirkland pers comm). Malaysian authorities were approached to fund some work on the virus at AAHL but declined, and it is doubtful much work has been done elsewhere.

Conclusions:

- **There is insufficient information available to make detailed recommendations on the disposal or disinfection of animal carcases or products exposed to Nipah virus.**

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Mohd Nor, M.N., Gan, C.H, & Ong, B.L. 2000, 'Nipah virus infection in peninsular Malaysia', *Rev sci tech Off int Epiz*, vol. 19(1), pp. 160-165.

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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 India[2] with the following thermal stability:

Temp (^{o}C)	Half-life
-20	24.2 days
4	9.9 days
37	3.3 hours
56	2.2 minutes

Lefevre (1987) reported comparable data, estimating a half-life of around two hours at 37^{o}C and inactivation within one hour at 50^{o}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 carcases stored at 4^{o}C (Lefevre 1987). No other references were found during this review regarding the persistence of PPRV in carcases or meat products. It is reasonable to assume that PPRV would be inactivated by the decline in pH accompanying rigor mortis. 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 carcases stored at 4^{o}C for 30 days, and from carcases aged for 24 hours and then kept at 4^{o}C for 8 days. The same paper reports recovery of virus from carcases 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).

[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.

Milk and milk products: No primary references were found on the presence or persistence of PPRV in milk. AUSVETPLAN (DPIE 1996a) is confusing in this regard, first extrapolating information from rinderpest to suggest that PPRV may infect sheep or goat milk, then stating unequivocally that the virus is present in milk.

If the virus is present in milk, prolonged survival is unlikely and pasteurisation should inactivate the virus, but this is unconfirmed (AQIS 1999b). AUSVETPLAN (DPIE 1996a) stipulates the heat treatment of milk for milk powder in the case of an outbreak.

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.

Conclusions:

- **The peste des petits ruminants virus is a fragile virus which is unlikely to survive for more than a few hours away from an animal host or animal products.**
- **PPR is principally spread by the respiratory route, with close contact required between infected and susceptible animals. However, recently contaminated fomites may also be a source of infection.**
- **Until more is known PPRV should be considered equivalent to Rinderpest virus that can survive in meat for up to a month. Carcases from infected flocks/herds should be rendered, burned or buried.**
- **Milk should be pasteurised.**
- **Salting of skins protects the virus from degradation. Fully processed skins and scoured wool are a negligible disease risk.**
- **Semen and embryos should not be used.**

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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. It is inactivated by most disinfectants, including phenol, chinosol, formalin, beta-propiolactone, trypsin, and 1M hydroxylamine. Strong alkalis are the most effective agents (Timoney *et al* 1988).

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 carcases. 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 carcases stored at 4°C for 30 days, and from carcases aged for 24 hours and then kept at 4°C for 8 days. The same paper reports recovery of virus from carcases 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).

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. DPIE (1996) 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 (DPIE 1996) stipulates the heat treatment of milk for milk powder from restricted and control areas in the case of an outbreak.

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). There appears to be a real but low risk of infectivity of semen or embryos.

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

Conclusions:

- **Rinderpest virus is a fragile virus which is unlikely to survive for more than a few hours away from an animal host or animal products. The virus is readily inactivated by exposure to heat, UV light and a wide range of disinfectants.**
- **Rinderpest is principally spread by the respiratory route, with close contact required between infected and susceptible cattle. However, recently contaminated fomites may also be a source of infection. Oral infection has been reported.**
- **Rinderpest virus can survive in meat for up to a month. Carcases from infected herds should be rendered, burned or buried.**
- **Milk from infected herds should be heat treated.**
- **Salting of hides protects the virus from degradation. Fully processed skins are a negligible transmission risk.**
- **Semen and embryos should not be used.**

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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 $\log_{10}$ at varying pH was:

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

For differing temperatures, the time to reduce infectivity by 1 $\log_{10}$ was:

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

(Woese 1960). Bengis (1997) notes that 69°C "appears to be the critical temperature". 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.

The relative lability of FMDV in alkaline conditions means that 2% NaOH or KOH and Na_2CO_3 can be used as cheap and effective disinfectants. Acids are also effective, but are more prone to neutralisation by organic materials such as blood and faeces (Thomson 1994).

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).

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).

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 carcase 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 carcase. It survived four months of freezing at -20^{o}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^{o}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^{o}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^{o}C for 30 seconds has been shown to reduce infectivity by $10^{5.4} – 10^{6.0}$ ID_{50} (Van Bekkum and de Leeuw 1978).

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. Survival of FMDV in dried milk has been demonstrated with only a 2 log reduction at 100^{o}C for 60 minutes (Nikitin and Vladimirov 1965). The effectiveness of heat treatment of viruses generally increases with increasing water activity.

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^{o}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/m^{3} ethylene oxide gas at 52^{o}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^{o}C, for 2 weeks at 18^{o}C, and for 2 days at 37^{o}C (McColl *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 be 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 provided that zona pellucida-intact embryos are used and are handled according to IETS recommendations. AQIS (1999b) and Sutmoller 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^{o}C to 1 hour at 55^{o}C in pig slurry, and between 5 weeks at 20^{o}C and >60 minutes (inactivation not reached) at 55^{o}C in cattle slurry. Initial concentration of virus was $10^{4.8}$ $TCID_{50}$/50uL in each case. Eizenberger (unpublished, cited in Haas *et al* 1995) demonstrated survival of FMDV in cattle slurry of 84 days at 4^{o}C to 70 days at 17^{o}C. AFFA (2001a) cites several figures for survival in manure, ranging from 6 to 42 days.

Conclusions:

- **FMD virus is a sensitive virus which, under normal conditions, does not survive for more than a few days away from an animal host or animal products. However, the virus can survive for months in a protected environment.**
- **The FMD virus is sensitive to changes in pH, heat and drying.**
- **Infection principally occurs by the respiratory and oral routes, with transmission by direct contact, windborne spread and fomites. The virus is highly contagious. Biosecurity of potentially infective material is very important.**
- **Carcases should be buried or burned on-site. Burial, burning and rendering off-site are less desirable options which should only be undertaken with a high level of biological security during transportation.**
- **Milk from infected herds should be heat treated. (Refer to the FMD AUSVETPLAN strategy for more specific information).**

- **Salting of skins protects the virus from degradation. Unprocessed skins that may be contaminated with FMD virus should be buried, burned or disinfected prior to further processing. Fully processed skins are a negligible disease risk.**
- **Wool that may be contaminated with FMD virus should be stored at 18ºC for four weeks or sent for industrial scouring. Scoured wool poses a negligible disease risk.**
- **Semen and embryos should be destroyed if collected less than one week prior to or at any time after the first case of FMD in the herd.**

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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.

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 carcases 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 carcases frozen at -20°C. The skin yielded 10^6 TCID (tissue culture infective doses) per gram, intercostal muscle 10^4 TCID, and rib bone and kidney cortex 10^3 TCID, similar to titres detected at the beginning of the storage period. Watson (1981) reported no drop in titre of SVDV in carcase material held for twelve months at 12-17°C.

A number of 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^{o}C".

SVDV survives for many months in buried carcases (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^{o}C. The temperature subsequently rose to 25^{o}C and the virus was not recovered.

Conclusions:

- **The swine vescicular disease virus is a persistent virus which can survive away from an animal host for months. It can survive a wide range of temperatures and pH, and is resistant to commonly used disinfectants.**
- **Infection principally occurs by the respiratory and oral routes, with spread by direct contact, fomites or ingestion of infected material. The virus is highly contagious. Biosecurity of potentially infective material is most important.**
- **Carcases should be burned, rendered or buried. Burial must be in a manner that precludes subsequent scavenging, at a site where there is no effluent discharge.**
- **Unprocessed skins that may be contaminated with SVD virus should be buried, burned or subjected to further processing. Fully processed skins are a negligible disease risk.**

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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 carcases 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), but 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 (unaerated). 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).

Conclusions:

- **The Teschen disease virus is a persistent virus which can survive away from an animal host for months. It can survive a wide range of temperatures and pH, and is resistant to commonly used disinfectants.**
- **Disease transmission occurs with an oral-faecal cycle.**

- **Meat and processed skins pose a negligible transmission risk.**

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Poxviridae

Lumpy skin disease

Agent:
Family Poxviridae, genus capripoxvirus

Agent type:
Virus

Persistence and inactivation:
General characteristics: Lumpy skin disease 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 closely related sheep pox virus is inactivated by heating at 56°C and is susceptible to UV light (Geering *et al* 1995).

LSDV is inactivated by a wide range of disinfectants, including the detergent SDS, ether, and chloroform (Weiss 1968).

The transmission of LSD 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 mains 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.

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 found in semen for up to 22 days (Davies 1991). Kahrs *et al* (1980) noted that the significance of semen in the transmission of the virus is not clear. No information was found on the presence of LSDV in embryos.

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.

Conclusions:

- **The lumpy skin disease virus is a persistent virus which can survive away from an animal host for months. It can survive a wide range of temperatures and pH, but is susceptible to commonly used disinfectants.**
- **LSD is mainly spread by biting insects, with mechanical transmission and a cattle-insect-cattle cycle.**
- **Unprocessed skins that may be contaminated with LSD virus should be buried, burned or disinfected prior to further processing. Fully processed skins are a negligible disease risk.**
- **While meat poses a negligible transmission risk, blood and other by-products are not. Therefore it is not practical to process carcasses at abattoirs.**
- **Milk from infected herds should not be used. Burial and burning are the preferred methods of disposal.**

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Sheep 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). Sheep pox virus has been shown to persist in vesicular fluid for 2-3 years stored at 0^oC or -15^oC (Drieux 1975).

Lumpy skin disease virus is reported to be stable over pH range 6.6-8.6 (Geering *et al* 1995). 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 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 $\log_{10}$ $TCID_{50}$/mL:

- 45^oC, two hours, 2.3 $\log_{10}$
- 50^oC, 30 minutes, 4 $\log_{10}$
- 50^oC, one hour, 6 $\log_{10}$
- 55^oC, 30 minutes, 4.6 $\log_{10}$
- 55^oC, one hour, virus not detectable
- 60^oC, 30 minutes, 5.6 $\log_{10}$
- 60^oC, one hour, virus not detectable
- 65^oC, five minutes, 5 $\log_{10}$
- 60^oC, 30 minutes, virus not detectable.

Other authors have shown similar results, although there are some strain differences (AQIS 1999).

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, DPIE 1996).

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 carcases are unlikely to pose an infective risk. The skins and wool, however, do pose a problem.

AUSTVETPLAN (DPIE 1996) 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).

AUSTVETPLAN (DPIE 1996) stipulates that milk from suspect animals is to be destroyed by heat, acid or 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.

AUSTVETPLAN (DPIE 1996) stipulates that any skins, wool or fibre which may have been contaminated must 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.

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).

Conclusions:

- **Sheep pox virus is a persistent virus which can survive away from an animal host for months.**
- **Infection mainly occurs by the respiratory route, either directly with close animal to animal contact or indirectly from the environment.**
- **Meat poses a negligible transmission risk, however the surface of fresh carcases may be contaminated with sheep pox virus. For this reason, fresh carcases and by-products should be buried or burned.**
- **AUSVETPLAN requires that skins and wool which may have been contaminated with sheep pox virus must be buried or burned. Fully processed skins are a negligible disease risk.**

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

The optimal pH for AHSV is 7.0-8.5, with the virus showing greater lability off the acid end of this range. It is resistant to lipid solvents such as ether.

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).

Carcases and meat products: No references were found on the persistence of AHSV in carcases 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 carcases 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: No references were found on the presence of AHSV in semen.

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

Conclusions:

- **The African horse sickness virus is a sensitive virus that is not able to survive for any length of time away from an animal host.**
- **African horse sickness is spread by *Culicoides* midges.**
- **Special disposal precautions are not necessary, except for freshly dead animals, which should be buried, burned or rendered.**
- **Animal products pose a negligible transmission risk and no special disposal measures are warranted.**

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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Bluetongue

Agent:
Family Reoviridae, genus orbivirus

Agent type:
Virus

Persistence and inactivation:
General characteristics: Bluetongue virus is very stable in blood and tissue specimens at 20°C, 4°C, and -70°C but not at -20°C. In the absence of extraneous protein it is extremely unstable at high temperatures. BTV is unstable below pH 6.5 and in the presence of 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 (with the exception of 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 carcases or meat products. The acid lability of BTV would suggest inactivation of the virus during post-mortem maturation of the carcase. AQIS (1999a) has determined that bluetongue "is unable to be transmitted by meat or meat products" and AUSVETPLAN (DPIE 1996) states 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 BTV.

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. 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 and Seidel 1990) gives 95% confidence that the percentage of BTV-contaminated embryos from viraemic donors would probably be not more than 1% (Sutmoller and Wrathall 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.

Conclusions:

- **Bluetongue is spread by *Culicoides* vectors. Transmission does not occur with direct contact between infected and susceptible animals in the absence of the vector. Nor does the virus spread by indirect means.**
- **Carcases and animal products pose a negligible transmission risk and no special disposal measures are warranted.**
- **Embryos collected from infected donors should be washed according to IETS standards before use. Semen collected from bulls during the infective phase should not be used.**

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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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) were able to reduce infectivity *in vitro* with 0.5% trypsin, or with exposure to pH 3.0 for one hour at 37^{o}C. The virus was totally inactivated after 5 minutes at 60^{o}C, with "considerable loss" of infectivity at 56^{o}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).

Conclusions:

- **Equine encephalosis virus is spread by arthropod vectors.**
- **Carcases and animal products derived from horses with equine encephalosis are unlikely to pose a transmission risk for any significant period after death. No special disposal measures are warranted.**

References:
Coetzer, J.A.W., & Erasmus, B.J. 1994, 'Equine encephalosis', in *Infectious diseases of livestock, Vol II*, eds. Coetzer, J.A.W., Thomson, G.R., & Tustin, R.C., Oxford University Press, Cape Town, pp. 476-479.

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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^{o}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 ID_{50}/mL in plasma stored at 4^{o}C for 24 hours. Rapid freezing / thawing of plasma at -70^{o}C caused a reduction in titre from 10^8 to between 10^3 and 10^4 ID_{50}/mL, a level which was maintained during storage at -70^{o}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.

Conclusions:

- **The Jembrana disease virus is a fragile virus that is not able to survive for any length of time away from an animal host.**
- **Jembrana disease is transmitted mechanically by biting insects. It may also spread iatrogenically, with mass vaccination or other intervention.**
- **Animal carcases and skins pose a negligible transmission risk. No special disposal measures are warranted.**
- **Milk from infected herds should be pasteurised.**

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.

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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^{o}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^{o}C and relatively resistant to repeated freezing and thawing (Petursson *et al* 1990). One $\log_{10}$ (90%) reduction in infectivity was reported at 50^{o}C in 1% serum (pH 7.3-7.5) after 10 minutes, and a 5 $\log_{10}$ reduction at 56^{o}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 carcases 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 in regard to milk, because the virus is present in monocyte/macrophage cells, although pasteurisation of goat milk at 56^{o}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^{o}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 as a result 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).

Conclusions:

- **The maedi-visna virus is a fragile virus that is not able to survive for any length of time away from an animal host.**
- **Transmission requires close contact between infected and susceptible animals, with the disease principally spread from dam to offspring via the colostrum or milk.**
- **Animal carcases and skins pose a negligible transmission risk. No special disposal measures are warranted.**
- **Milk from infected herds should be pasteurised.**

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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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).

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

Carcases and meat products: No primary information is available on the survival of PAV in carcases 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 carcase 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.

Conclusions:

- **The pulmonary adenomatosis virus is a fragile virus that is not able to survive for any length of time away from an animal host.**
- **Disease spread occurs by the respiratory route, with close contact required between infected and susceptible animals.**
- **Animal carcases and products pose a negligible transmission risk. No special disposal measures are warranted.**

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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 the course of this review (the absence of any information was confirmed by McColl, pers comm and Field, pers comm).

Conclusions:

- **Little is known about the persistence of Australian lyssavirus. It is likely to have similar properties to rabies virus, but this has not been confirmed. Rabies is a fragile virus that cannot survive for long away from an animal host and does not persist in carcases or animal products.**

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.

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Rabies

Agent:
Family Rhabdovirus, genus lyssavirus

Agent type:
Virus

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

RV is susceptible to UV light and to lipid solvents (soapy water, ether, chloroform, acetone), ethanol 45-75%, quaternary ammonium compounds (e.g. 0.2% cetrimide), 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 carcases or meat products were found during this review, and MacDiarmid and Thompson (1997) state that rabies virus has never been isolated from meat.

Wandeler (pers comm) advised that the fragility of rabies viruses has "probably precluded detailed studies of their inactivation in carcases". There is an assumption that virus on the exposed surfaces of the carcase 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).

Fooks (pers comm) has confirmed the paucity of data on persistence of rabies in carcases and animal products, and speculated that the virus "would not survive for long (a few weeks)". He understood that rendering any carcase 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 for any period of time 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.

Conclusions:

- **Rabies is a fragile virus that is readily inactivated by exposure to heat, UV light, lipid solvents and commonly used disinfectants. The virus cannot survive for long away from an animal host and does not persist in carcases or animal products.**
- **Special disposal precautions are not necessary, except for freshly dead animals, which should be buried, burned or rendered.**

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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 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^{o}C but can survive in soil at 4-6^{o}C (Hanson and McMillan 1990). VSV may be preserved for years at -60^{o}C and by freeze-drying under vacuum (Hanson 1981).

The virus survives for 3-4 days in infected saliva on buckets, feed racks and hay (Hanson 1952). It is rapidly inactivated by all common disinfectants, including formalin, phenols and quaternary ammonium compounds (Hanson and McMillan 1990).

The epidemiology of VS is poorly understood. 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^{o}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).

Conclusions:

- **The vesicular stomatitis virus is a sensitive virus which, under normal conditions, does not survive for more than a few days away from an animal host.**
- **The epidemiology of VS is poorly understood. Animals are thought to become infected only via insect bites or abrasions to the skin or mucus membranes.**
- **Fresh carcases should be disposed of in a manner that prevents insect attack or scavenging.**
- **Meat and skins pose a negligible transmission risk. No special disposal measures are warranted.**
- **Milk from infected herds should be pasteurised.**

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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, and 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).

Conclusions:

- **The Getah virus is a fragile virus that cannot survive for long away from an animal host and does not persist in carcases or animal products.**
- **Getah virus is spread by mosquitoes.**
- **Carcases and animal products pose a negligible transmission risk. No special disposal precautions are warranted.**

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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).

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

The half life was decreased by rapid changes in pH.

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. 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 carcases or animal products, and none were uncovered during this review.

Carcases 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 ^{0}C and at least one month when stored at -20 ^{0}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.

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).

Conclusions:

- **The PRRS virus is a sensitive virus that can survive in the environment for up to three weeks, and longer in chilled and frozen meat products.**
- **Transmission of PRRS occurs principally by respiratory aerosol, with windborne spread a feature of the disease. Oral infection can also occur.**
- **Fresh carcases should be disposed of in a manner that prevents scavenging. Meat is a risk and should be cooked for at least 70^{0}C for 11 minutes. Fresh carcases should be processed to at least this time/temperature or be buried, rendered or burnt.**
- **Skins pose a negligible transmission risk, and no special disposal precautions are warranted.**
- **Semen collected from infected boars should not be used.**

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Western, Eastern and Venezuelan equine encephalomyelitis

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.

References to the stability and inactivation of the virus were difficult to find – emphasis has clearly been placed on trying to preserve the organism for diagnostic purposes rather than to destroy it. Monath and Trent (1981) state that serum or tissue suspensions should be preserved at -70^{o}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).

Conclusions:

- **The equine encephalomyelitis viruses are fragile viruses that are not able to survive for any length of time away from an animal host.**
- **The disease is transmitted by mosquitoes.**
- **Animal carcases and products pose a negligible transmission risk. No special disposal measures are warranted.**

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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 safely sterilised after 30 minutes at 60°C).

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).

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).

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).

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^{o}C and for 112 days at 4^{o}C in slurry under simulated field conditions.

Conclusions:

- **The African swine fever virus is a persistent virus which can survive away from an animal host for over a year under the right conditions.**
- **Infection occurs mainly by the respiratory and oral routes, either directly with close animal to animal contact or indirectly from the environment. Blood-sucking insects can also spread the disease.**
- **The ASF virus is highly contagious. Biosecurity of potentially infective material is important.**
- **Fresh carcases and meat from infected animals should be buried, burned or rendered. If buried, the burial site must preclude scavenging by feral pigs. Movement of carcases off-site for disposal should only be undertaken with a high level of biological security during transportation.**
- **Unprocessed skins that may be contaminated with ASF virus should be buried, burned or disinfected prior to further processing. Fully processed skins are a negligible disease risk.**
- **Semen and embryos collected from infected animals should be destroyed.**

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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). It 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).

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 carcases of infected animals. ADV was recovered from the carcase 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 carcases, provided that maximum temperatures in the pile exceed 60°C.

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 for any period of time 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 Aujeszky's disease]".

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.

Faeces: A number of authors have studied the survival of ADV in pig and cattle slurry. Bøtner (1991) reported findings reasonably consistent with previous studies. Inactivation increased with temperature, requiring 15 weeks at 5^{o}C, 2 weeks at 20^{o}C, 5 hours at 35^{o}C and 10 minutes at 55^{o}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^{o}C) for unidentified reasons. Muller (1973) reported that ADV may survive for 3-15 weeks.

Conclusions:

- **Aujeszky's disease virus is a sensitive virus which, under normal conditions, does not survive for more than a few days away from an animal host. It is readily inactivated by drying, heat, UV light and commonly used disinfectants.**
- **Infection principally occurs by the respiratory and oral routes, with close contact required between infected and susceptible animals. Transmission can also occur with infected semen.**
- **The over-riding consideration with disposal of fresh carcases is that infective material must not be consumed by pigs. Fresh carcases from infected animals should be buried, burned or rendered. If buried, the burial site must preclude scavenging.**
- **The meat from clinically healthy animals on affected farms may be processed normally.**
- **Semen collected from infected animals should not be used.**

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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 carcases 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.

Conclusions:

- **Little is known about the persistence of Borna disease virus in either the environment or in animal products, and the epidemiology of the disease is poorly understood.**
- **The risk of disease transmission with animal products appears low.**

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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).

Over a number of studies CSFV has been shown to be stable at neutral to slightly alkaline values (around pH 5-10). It is rapidly inactivated below pH 3 and above pH 10 (Edwards 2000).

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^{o}C, -30^{o}C, or -80^{o}C, but inactivated the virus after 30 minutes at 56^{o}C or 10 minutes at 60^{o}C. Inactivation took one minute at 90^{o}C, two minutes at 80^{o}C and five minutes at 70^{o}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).

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).

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).

In over 100 cases of CSF managed in Lower Saxony, Germany, between 1993 and 1996, no outbreaks were connected to the transport of carcases 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).

Carcases 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^{o}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^{o}C, 15 minutes at 69^{o}C, or one minute at 71^{o}C (Farez and Morley 1997 and Edwards 2000, citing several authors). Heating for 30 minutes at 62.5^{o}C failed to inactivate the virus so temperature control is critical (Edwards 2000).

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.

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^{o}C to instantaneous at 50^{o}C (detection limit 0.7 $\log_{10}$ $TCID_{50}$/50L). Eizenberger (unpublished, cited in Haas *et al* 1995) found CSFV surviving for at least 70 days at 17^{o}C and for 84 days at 4^{o}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.

Conclusions:

- **The classical swine fever virus is a sensitive virus which, under normal conditions, does not survive for more than a few days away from an animal host or animal products. However, the virus can survive for a few weeks in a cool, moist protein-rich environment. It can survive for months in chilled pig meat and for years in frozen pig meat.**

- **Infection principally occurs by the respiratory and oral routes, with transmission by direct contact or swill feeding.**

- **All animal products potentially contaminated with the virus should be regarded as a transmission risk.**

- **The over-riding consideration with disposal of fresh carcases is that infective material must not be consumed by pigs.**
- **On-site burning is the preferred method for disposal of carcases and animal products.**
- **Burial, burning and rendering off-site are less desirable options, which should only be undertaken if transportation is biologically secure and scavenging by feral pigs and rodents is precluded.**

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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).

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).

On the basis of 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).

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

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 $10^{5.5}$ $TCID_{50}$/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).)

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. On the basis of 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).

Conclusions:

- **Infectious bursal disease virus is a persistent virus that can survive in the environment for months.**
- **Infection occurs by the respiratory and oral routes, with transmission by direct contact and fomites. The virus is highly contagious. Biosecurity of potentially infective material is very important.**
- **Burning, rendering and burial are the preferred methods for disposal of infective material.**

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Transmissible gastroenteritis

Agent:
Family Coronaviridae

Agent type:
Virus

Persistence and inactivation:
General characteristics: Transmissible gastroenteritis virus 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).

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).

TGE 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 TGE 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 *et al* (1991) demonstrated transmission from apparently healthy animals from a TGE-endemic area. TGE virus was found in tonsils from 4 of 500 pigs but not from lymph nodes or muscle. The study showed the risk of introducing TGE through pig carcases 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 TGE. In a draft assessment, AFFA (2001b) have concluded that skins and hides pose an insignificant risk in the transmission of TGE.

Semen/embryos: There are no reports of TGE virus 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 considered to be 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^{o}C (Young *et al* 1955). Faeces containing 10^5 TGEV was inactivated within 6 hours when exposed to direct sunlight (DPIE 1996).

Conclusions:

- **The TGE virus is a sensitive virus which, under normal conditions, can survive in the environment for only a few days. It is inactivated by commonly used disinfectants.**
- **TGE is highly contagious. The disease has an oral-faecal cycle.**
- **Fresh carcases must be disposed of in a manner that precludes scavenging by pigs.**
- **Provided there is no swill feeding of pigs, meat and skins pose a negligible transmission risk. No special disposal precautions are warranted.**

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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 carcases 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.

Conclusions:

- **The vesicular exanthema virus is a persistent virus which can survive for several weeks away from an animal host or animal products. The virus can survive for months in chilled pig meat and for years in frozen pig meat.**
- **Infection occurs mainly by the oral route, with transmission by direct contact or swill feeding.**
- **All animal products potentially contaminated with the virus should be regarded as a transmission risk.**
- **The over-riding consideration with disposal of carcases and animal products is that infective material must not be consumed by pigs. Burial, burning and rendering are all suitable methods of disposal. If infective material is buried, the site must preclude scavenging by feral pigs.**

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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, Creuzfeld-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 (ID_{50}) 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). 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^{o}C for one hour (drying of tissue is known to enhance its thermostability)
- autoclaving, although gravity-displacement autoclaving at 132^{o}C for 4.5 hours has been recommended, as has porous-load autoclaving at 134-138^{o}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^{o}C for 30-60 minutes plus 1M or 2M NaOH).

Brown (1998) stated that, in regard to 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 carcases, 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".

Carcases 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^{o}C to 40^{o}C (average 3^{o}C to 25^{o}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 Norska 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 carcases 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 years[3]. 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 carcases 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 carcases 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

[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.

slaughtered could potentially include pre-clinical cases of BSE. The ash will therefore be incinerated as a precaution (Jeffrey pers comm).

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). A number of 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.

Conclusions:

- **The TSE diseases are caused by prions, which are extremely hardy. Prions can suvive in the environment for many years and are resistant to all conventional methods of disinfection.**
- **Infection occurs by the oral route, with an exceedingly small infective dose.**
- **Carcasses should be burned completely and residual ash collected, mixed with agricultural lime and deep buried at a suitable site which will need to be monitored and scavengers excluded.**
- **When burning is not practical, carcasses and other materials not able to be decontaminated, should be deep buried with caustic materials at a suitable site which will need to be monitored and scavengers excluded.**

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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 CO_2 and temperatures below 20^{o}C, and only takes place under appropriate conditions, most notably when an infected carcase is opened. Germination of spores occurs at 20-40^{o}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^{o}C dry heat for 30 minutes. Spores are much more robust, requiring 140^{o}C dry heat for up to 3 hours for inactivation (Buxton and Fraser 1977). Moist heat destroys spores at 100-115^{o}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).

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^{o}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^{o}C (Whitford 1978).

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

Early identification and disposal of carcases prior to opening helps to minimise environmental contamination. Carcases should be incinerated intact, or buried in a 2 metre deep grave, covered with one part of chloride of lime (at least 25% active chlorine) to three parts soil (de Vos 1994). 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 carcases after burning.

Bone meal contaminated with anthrax was found to be infective after 15 minutes of steam treatment at 115^{o}C degrees or 3 hours with dry heat at 140^{o}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 ton capacity)
- benzene vapour at 95-115^{o}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^{o}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 carcases 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^{o}C for 20 minutes, dry heated at 95^{o}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^{o}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 lime (at least 25% active chlorine) to three parts soil prior to filling in the hole.

Conclusions:

- **The body of an animal with anthrax contains vast numbers of anthrax bacteria. The bacteria can survive for only a few days in an intact carcase. However, on exposure to air, anthrax bacteria produce spores, which are very hardy and can survive in the environment for many years.**
- **Opening the carcase must be avoided at all costs.**
- **Burning is the preferred method of carcase disposal. When handling infected carcases it is important to avoid rupturing a carcase and/or spillage of body fluids.**
- **Surface decomposition is a less desirable option. Carcases managed this way must be sprayed with 5% formaldehyde and securely protected from scavengers.**
- **Deep burial is the least desirable disposal option, because anthrax spores can survive in the soil for decades. If carcases are deep buried, they must be at least two metres beneath the surface and liberally covered with quicklime.**
- **Skins and hides from anthrax infected animals are a significant disease risk. They should be disposed of in a similar maner to fresh carcases.**
- **Meat from animals clinically healthy at ante-mortem inspection is a negligible transmission risk. No special disposal procedures are warranted.**
- **Milk from affected herds should be pasteurised.**

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Heartwater

Agent:
Cowdria ruminantium

Agent type:
Bacterium

Persistence and inactivation:
Cowdria 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. *C. 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 *C. ruminantium* is heat labile and loses its viability within 12-38 hours at room temperature (Uilenberg pers comm). Alexander (1931) was able to maintain *C. ruminantium* in defibrinated blood for up to 38 hours. He observed that *C. 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 *C. ruminantium*. Infected ovine spleen and blood homogenates kept at -76^{o}C maintained infective virus for at least 2 years (Neitz 1968). Logan (1987) reported effective preservation of *C. ruminantium* at -70^{o}C to -196^{o}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 *Cowdria ruminantium* in carcases or animal products. As Uilenberg (pers comm) has pointed out, this is partly due to the extreme difficulty in proving whether or not the organism is alive.

Conclusions:

- ***C. ruminantium* is an obligate intracellular parasite that is spread by ticks.**
- **Carcases and animal products pose a negligible transmission risk. No special disposal precautions are warranted.**

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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. *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).

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 carcases (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 carcases 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^{o}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^{o}C, and at all time / temperature combinations down to 5 seconds / 65^{o}C. Survival in whey was less than 4 days at 17-24^{o}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).

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 (Verger 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, Verger 1980).

Verger (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.

Conclusions:

- ***B. abortus* is a sensitive bacterium that, under favourable conditions, can survive in the environment for several weeks to months. It is readily inactivated by heat, UV light and commonly used disinfectants.**
- ***B. abortus* is excreted in all body fluids, with the greatest number of organisms in uterine discharges, aborted foetuses, foetal membranes and milk. Infection occurs with ingestion, inhalation or contact with the conjunctiva or abraded skin.**
- ***B. abortus* was eradicated from Australia with a program that focused on the diagnosis of infected cattle, with little emphasis on carcase disposal or animal products.**

- **Carcases and animal products are not important in the epidemiology of the disease. However, they are important from a human health perspective – most human cases occur as a result of handling aborted foetuses or foetal membranes, or from consuming unpasteurised milk or milk products from infected cattle.**
- **Meat derived from affected herds is a negligible transmission risk.**
- **Milk from affected herds should be pasteurised.**
- **Semen should be destroyed. Embryos should be washed according to IETS standards.**

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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 similar to 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.

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^{o}C
- between 5 and 7.5 minutes at 61.1^{o}C
- less than 5 minutes at 62.8^{o}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.

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 carcases or meat products. *B. abortus* has been shown to survive up to 44 days in guinea pig carcases 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 carcases 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.

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 is 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^{o}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).

Conclusions:

- ***B. melitensis* is a relatively sensitive bacterium that, under favourable conditions, can survive in the environment for several weeks to months. It is readily inactivated by heat, UV light and commonly used disinfectants.**
- ***B. melitensis* is excreted in all body fluids, with the greatest number of organisms in uterine discharges, aborted foetuses, foetal membranes and milk. Infection occurs with inhalation, ingestion or contact with the conjunctiva or abraded skin.**
- **Carcases and animal products are not important in the epidemiology of the disease. However, they are important from a human health perspective – most human cases occur as a result of handling aborted foetuses or foetal membranes, or from consuming unpasteurised milk or milk products from infected sheep or goats.**
- **Meat derived from affected herds is a negligible transmission risk.**

- **Milk from affected herds should be pasteurised.**
- **Semen and embryos should be destroyed.**

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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 by virtue 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).

A number of 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.

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 carcases 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 carcases produced similar results, showing survival times of 2-4 weeks. Inactivation is aided by the decomposition of the carcase, 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 carcases 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^{o}C below those formerly identified for the *M. avium – M. intracellulare* complex. A one log reduction required approximately 25 minutes / 53^{o}C to one minute / 61^{o}C. A five log reduction required 350 minutes / 53^{o}C to one minute / 68^{o}C (all approximate figures read from graph). Ultraviolet radiation, amphyl 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 carcases into an animal digester, with pure cultures of various micro-organisms in dialysis bags also placed in the digester. The carcases were covered by hot alkaline solution and kept at 110-120^{o}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^{o}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 (10^{7} 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^{o}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^{o}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).

Conclusions:

- ***Mycobacterium bovis* is a sensitive organism which, under favourable conditions, can survive in the environment for a few weeks.**
- **Transmission between cattle is mainly by respiratory aerosol. Most human cases occur as a result of consuming unpasteurised milk from infected cattle.**
- **Fresh carcases should be disposed of in a manner that prevents scavenging.**
- **Meat and skins from affected herds pose a negligible transmission risk. No special disposal precautions are warranted.**
- **Milk from affected herds should be pasteurised.**

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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).

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 carcases, for a few days. Bacteria have been found at 10^{ll} – 10^{12} CFU/mL of blood of carcases 20-21 hours after death. Carcases may be a source of infection during these few days, and the dumping of carcases 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.

Conclusions:

- ***Pasteurella multocida* is a sensitive bacterium which, under favourable conditions, can survive in the environment for only a few days.**
- **Transmission between cattle is mainly by respiratory aerosol.**
- **Carcases and animal products pose a negligible transmission risk. No special disposal precautions are warranted.**
- **Semen should be destroyed.**

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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Glanders

Agent:
Burkholderia mallei

Agent type:
Bacterium

Persistence and inactivation:
General characteristics: *B. mallei* may 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).

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 and for about six weeks in contaminated stables.

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 carcases burnt or buried.

Skins, hides and fibres: AFFA (2001) has determined that there is some risk of at *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).

Conclusions:

- ***B. mallei* is a sensitive bacterium which, under favourable conditions, can survive in the environment for a few weeks. It is readily destroyed by exposure to sunlight and commonly used disinfectants.**
- ***B. malleis* is excreted in nasal discharges, urine, saliva, faeces and pus from infected animals. Infection occurs by ingestion or contamination of abraded skin.**
- **Fresh carcases should be disposed of in a manner that precludes scavenging.**
- **Meat and processed skins pose a negligible transmission risk. No special disposal precautions are warranted.**

References:

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

Battelli, C., Contento, F., Corsalini, T., Goffredo, G., Lazari, P., Puccini, V., & Sobrero, L. 1973, 'Glanders in a group of lions in captivity', *Veterinaria Italiana*, vol. 24, pp. 87-112, 113-116, cited in 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^{o}C and two-fold at 4^{o}C, but did not deteriorate at -70^{o}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^{o}C to less than one minute at 50^{o}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 exposure to chlorhexidine diacetate (2%) or alkyldimethylbenzylammonium chloride (10%) (Henton 1994).

Carcases and meat products: No studies on the survival of *T. equigenitalis* in carcases 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.

Conclusions:

- ***T. equigenitalis* is a fragile bacterium which, under normal conditions, can survive no longer than a week or two in the environment.**
- **Disease spread occurs by venereal means.**
- **Carcases and animal products pose a negligible transmission risk. No special disposal precautions are warranted.**
- **The semen from infected stallions should be destroyed.**

References:

Henton, M.M. 1994, 'Taylorella equigenitalis infection', in *Infectious diseases of livestock, Vol II,* eds. Coetzer, J.A.W., Thomson, G.R., & Tustin, R.C., Oxford University Press, Cape Town, pp. 1510-1513.

Sahu, S.P., Dardiri, A.H., Rommel, F.A., & Pierson, R.E. 1979, 'Survival of contagious equine metritis bacteria in transport media', *Am J Vet Res,* vol. 40, pp. 1040-1042.

Sahu, S.P., & Dardiri, A.H. 1980, 'Contagious equine metritis: isolation and characterization of the etiologic agent', *Am J Vet Res,* vol. 41, pp. 1379-1382.

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Timoney, P.J., Ward, J., McArdle, J.F., & Harrington, A.M. 1979, 'Thermal death times of the organism of contagious equine metritis 1977', *Vet Rec,* vol. 104, p. 530.

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Potomac fever

Agent:
Ehrlichia risticii

Agent type:
Bacterium

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 carcases or in animal products.

Conclusions:

- ***Ehrlichia risticii* is an obligate intracellular parasite that is spread by ticks.**
- **Carcases and animal products pose a negligible transmission risk. No special disposal precautions are warranted.**

References:
Holland, C.J. 1990, 'Biologic and pathogenic properties of *Ehrlichia risticii*: the etiologic agent of equine monocytic ehrlichiosis', in *Current topics in veterinary medicine and animal science. Vol. 54. Ehrlichiosis: a vector-borne disease of animals and humans*, eds. Williams, J.C., & Kakoma, I., Kluwer Academic Publishers, Netherlands, pp. 68-77.

Jenny, A.L. 1984, 'Potomac horse fever – National Veterinary Services Laboratories (USDA) report', *Am Assoc Eq Practnr Newsltr*, no. 2, pp. 64-66, cited in Holland 1990.

Palmer, J.E. 1987, 'Potomac horse fever', in *Current therapy in equine medicine*, ed. Robinson, N.E., W.B. Saunders Company, Philadelphia, pp. 92-93.

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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 as a result of both desiccation and UV light in a study by Windsor and Masiga (1977).

The organism is inactivated within 60 minutes at 50^{o}C and within two minutes at 60^{o}C, but may persist for at least twelve months in frozen lung tissue (Schneider *et al* 1994). 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^{o}C (Hudson 1968).

M. mycoides subsp *mycoides* SC is sensitive to 1% phenol (three minutes to inactivation), 0.05% 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).

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 carcases 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.

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).

Conclusions:

- ***M. mycoides* is a sensitive organism which, under favourable conditions, may survive in the environment for up to two weeks.**
- **Transmission between cattle is by respiratory aerosol.**
- **Carcases and animal products pose a negligible transmission risk. No special disposal precautions are warranted.**

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, Import risk analysis: Importation of dairy products for human consumption, AQIS, Canberra.

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

Hudson, J.R. 1968, *Aust Vet J*, vol. 44, pp. 123-129, cited in Windsor and Masiga 1977.

Hyslop, N. St G. 1959, *Proc 16th Int vet Congress*, pp. 535-538, cited in Windsor and Masiga 1977.

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Nicholas, R., Bashiruddin, J., Ayling, R., & Miles, R. 2000, 'Contagious bovine pleuropneumonia: a review of recent developments', *Vet Bull*, vol. 70, pp. 827-838.

Provost, A., Perreau, P., Breard, A., Le Goff, C., Martel, J.L., & Cottew, G.S. 1987, 'Contagious bovine pleuropneumonia', *Rev sci tech Off int Epiz*, vol. 6, pp. 625-679, cited in Schneider *et al* 1994.

Schneider, H.P., van der Lugt, J.J., & Hubschle, O.J.B. 1994, 'Contagious bovine pleuropneumonia', in *Infectious diseases of livestock, Vol II*, eds. Coetzer, J.A.W., Thomson, G.R., & Tustin, R.C., Oxford University Press, Cape Town, pp. 1485-1494.

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.

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Epizootic lymphangitis

Agent:
Histoplasma capsulatum var *farciminosum*

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 (^{o}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 carcases.

Conclusions:

- ***H.capsulatum* is a persistent organism which can survive in the environment for several months.**
- **Given the persistence of *H. capsulatum* and potential for spread by flies and fomites, burial or burning of infected carcases and contaminated animal products appears warranted.**

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.

Bardelli, P., & Ademollo, A. 1927, 'Sulla resistenza delle conture di *Cryptococcus farciminosus.* Rivolta agli agenti fisici e chimici', *Annual Igiene*, vol. 37, pp. 81-85, cited in Al-Ani 1999.

Gabal, M.A., & Hennager, S. 1983, 'Study on the survival of *Histoplasma farciminosum* in the environment', *Mykosen*, vol. 26, pp. 481-484, 487.

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.

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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^{o}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 carcases. 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 carcases 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.

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 carcases 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.

Conclusions:

- ***T. evansi* is a fragile organism which, under normal conditions, is unlikely to survive away from an animal host for more than three days.**
- **Spread between mammalian hosts mainly occurs with biting insects. However, transmission can also spread with ingestion of an infected fresh carcase.**
- **Fresh carcases should be disposed of so as to preclude scavenging.**
- **Meat and other animal products pose a negligible transmission risk. No special disposal precautions are warranted.**
- **Semen should not be used.**

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 *Trypansomoa evansi, Theileria mutans, Babesia bigemina* and *Anaplasma marginale* following long-term maintenance at -70^{o}C', *Arch Inst Razi*, vol. 32, pp. 47-50.

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).

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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 carcases or meat products. It seems reasonable to extrapolate from the study by Sarmah (1998) on survival of *T. evansi* in rat carcases, 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 trypanosomiases.

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.

Conclusions:

- ***T. equiperdium* is a fragile organism which, under normal conditions, is unlikely to survive away from an animal host for more than three days.**
- **Disease spread occurs mainly by venereal means. Foals may possibly become infected from contact with vaginal discharges or ingestion of infected milk.**

- **Carcases and animal products pose a negligible transmission risk. No special disposal precautions are warranted.**
- **Semen should not be used.**

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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East Coast fever

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 if infected ticks (Lawrence *et al* 1994).

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.

Conclusions:

- ***T. parva parva* is a fragile organism which, under normal conditions, is unlikely to survive away from an animal host or tick for more than two days.**
- **Ticks are required for disease spread.**
- **Carcases and animal products pose a negligible transmission risk. No special disposal precautions are warranted.**

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.

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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.

Conclusions:

- ***Babesia equi* and *Babesia caballi* are fragile organisms which, under normal conditions, are unlikely to survive from an animal host or tick for more than two days.**
- **Ticks are required for disease spread.**
- **Carcases and animal products pose a negligible transmission risk. No special disposal precautions are warranted.**

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.

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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^{o}C, and 15-18 days at 2-9^{o}C. The closely related species *P. caniculi* from rabbits appeared to have significantly lower survival below a relative humidity of 65-75% at 30^{o}C.

P. ovis is susceptible to a number of 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 carcases might be expected to fall within the range of estimates described above, but no specific studies on survival on carcases 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.

Conclusions:

- ***P. ovis* is a sensitive mite which, under natural conditions, is unlikely to survive for more than three weeks away from an animal host.**
- **Close physical contact is required for transmission.**
- **Processed skins, scoured wool and other animal products pose a negligible transmission risk.**

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.

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.

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Tropilaelaps mite

Agent:
Tropilaelaps clareae

Agent type:
Mite

Persistence and inactivation:
AUSVETPLAN (DPIE 1996) notes that *Tropilaelaps clareae* persists only in a hive with adult bees and live brood. Adult mites do not survive more than 2-4 days away from bee brood. Gravid females survive only a short period on the adult bee, because their unspecialised chelicerae cannot pierce the integument of the host. They die within two days unless they can deposit their eggs (Sammataro *et al* 2000).

The literature contains numerous references to control of *T. clareae* populations using chemotherapy. Control with chemotherapeutic agents is difficult, because many of the mites are physically partitioned from the chemical, necessitating multiple treatments over several weeks to combat the ongoing emergence of parasites from the brood (Burgett and Kitprasert 1990).

A number of treatments have been shown to reduce mite populations, including formic acid, sulphur and chlorobenzilate (Sammataro *et al* 2000). A slow-release fluvalinate formulation appears to be the preferred treatment (Burgett and Kitprasert 1990).

Conclusions:

- **AUSVETPLAN stipulates the destruction of infected hives by either burning or treatment with an insecticide or solvent followed by burial.**
- **Given the low unit value of bees and their products and the relative ease of disposal, there is no obvious reason to consider lower cost, higher risk disposal methods.**

References:
DPIE (Department of Primary Industries and Energy) 1996, *Australian Veterinary Emergency Plan, AUSVETPLAN Edition 2.0, Disease Strategy Bee diseases*, DPIE, Canberra.

Burgett, D.M., & Kitprasert, C. 1990, 'Evaluation of Apistan as a control for *Tropilaelaps clareae* (Acari: Laelapidae), an Asian honey bee brood mite parasite', *Am Bee J*, vol. 130, pp. 51-53.

Sammataro, D., Gerson, U., & Needham, G. 2000, 'Parasitic mites of honey bees: life history, implications, and impact', *Annu Rev Entomol*, vol. 45, pp. 519-548.

Varroa mites

Agent:
Varroa destructor and Varroa jaobsoni

Agent type:
Mite

Persistence and inactivation:
Varroa destructor has only recently been described as a separate species from *Varroa jacobsoni* after Anderson and Trueman (2000) demonstrated significant genetic variation. *Varroa jacobsoni* reproduces only on *Apis cerana* and is thus not a serious problem for managed honey bee colonies (*Apis mellifera*). Two haplotypes of *V. destructor* cause significant damage to *A. mellifera* (Anderson and Trueman 2000). The literature prior to this time therefore contains a mixture of descriptions of both species under the name *V. jacobsoni* (Anderson pers comm).

Varroa mites are transmitted when beekeepers transfer colonies or brood between colonies, or when bees rob each other or meet at flowers. The feral bee population may act as a reservoir of infection (Anderson pers comm).

As with the other mites of bees considered in this review, *Varroa* spp are obligate ectoparasites (Anderson and Trueman 2000). Varroa can survive off the host for 18-70 hours (Sammataro *et al* 2000). Anderson (1997) found that adult female *V. jacobsoni* did not survive longer than 9 days in leafcutter bee nest material at 5^{o}C. AUSVETPLAN states that mites will die in three days without food (DPIE 1996). Anderson (1994), examining the survival of adult female mites in the presence or absence of *A. cerana* and *A. mellifera* worker brood, reported survival of mites away from pupae for up to 96 hours.

A slow-release fluvalinate formulation appears to be the preferred treatment for *Varroa* spp, although resistance to this chemical has been recorded since 1995 in Italy. Other treatments include coumaphos, formic acid, oxalic and lactic acids (Sammataro *et al* 2000). The effectiveness of a gel formulation of thymol has recently been demonstrated (Mattila and Otis 1999).

Destruction of bees, combs and hive components as well as feral bee colonies where present is the only method for eradication, and would need to be instituted very quickly in case of an incursion.

Conclusions:

- **AUSVETPLAN stipulates the destruction of infected hives by either burning or treatment with an insecticide or solvent followed by burial.**
- **Given the low unit value of bees and their products and the relative ease of disposal, there is no obvious reason to consider lower cost, higher risk disposal methods.**

References:

Anderson, D.L. 1994, 'Non-reproduction of *Varroa jacobsoni* in *Apis mellifera* colonies in Papua New Guinea and Indonesia', *Apidologie*, vol. 25, pp. 412-421.

Anderson, D.L. 1997, *Evaluation of quarantine risks associated with importations of the Leafcutter Bee (*Megachile rotundata) *to Australia from Canada. Report on a consultancy conducted in Canada and the United States from 30 June to 15 July 1997.* CSIRO Division of Entomology, Canberra.

Anderson, D.L., & Trueman, J.W.H. 2000, '*Varroa jacobsoni* (Acari: Varroidae) is more than one species', *Exp Appl Acarol,* vol. 24, pp. 165-189.

DPIE (Department of Primary Industries and Energy) 1996, *Australian Veterinary Emergency Plan, AUSVETPLAN Edition 2.0, Disease Strategy Bee diseases,* DPIE, Canberra.

Mattila, H.R., & Otis, G.W. 1999, 'Trials of Apiguard, a thymol-based miticide. Part 1. Efficacy for control of parasitic mites and residues in honey', *Am Bee J,* vol. 139, pp. 947-952.

Sammataro, D., Gerson, U., & Needham, G. 2000, 'Parasitic mites of honey bees: life history, implications, and impact', *Annu Rev Entomol,* vol. 45, pp. 519-548.

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Acarapis (tracheal) mite

Agent:
Acarapis woodi

Agent type:
Mite

Persistence and inactivation:
Acarapis woodi is an obligate parasite that primarily infests the tracheae and air sacs of adult honey bees (*Apis mellifera*). Mites disperse by questing on bee setae when the host bee is 15-25 days old. Survival of mites may not exceed a few hours during this period off the host, as mites are very sensitive to desiccation and starvation. Ambient temperature, humidity and state of nourishment are critical factors in the survival time (Sammataro *et al* 2000).

A. woodi poses problems for chemical control, because suitable treatments must reach bee tracheae via a volatile compound, be inhaled by the bee, and be lethal only to the mite. Residues in bee products must also be avoided. The only product registered in the United States is pure menthol crystals. Amitraz and formic acid are also used as treatments elsewhere (Sammataro *et al* 2000). Flumethrin, fluvalinate, and methyl palmitate can be used to reduce mite populations. Residues in honey and beeswax have been identified following chemotherapy.

Conclusions:

- **AUSVETPLAN stipulates the destruction of infected hives by either burning or treatment with an insecticide or solvent followed by burial.**
- **Given the low unit value of bees and their products and the relative ease of disposal, there is no obvious reason to consider lower cost, higher risk disposal methods.**

References:
DPIE (Department of Primary Industries and Energy) 1996, *Australian Veterinary Emergency Plan, AUSVETPLAN Edition 2.0, Disease Strategy Bee diseases*, DPIE, Canberra.

Sammataro, D., Gerson, U., & Needham, G. 2000, 'Parasitic mites of honey bees: life history, implications, and impact', *Annu Rev Entomol,* vol. 45, pp. 519-548.

Screw-worm fly

Agent:
Chrysomya bezziana, Cochliomyia hominivorax

Agent type:
Fly

Persistence and inactivation:
Screw-worm flies 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^{o}C, will not move at <10^{o}C and may not mate at 10-16^{o}C. It does not survive in areas that experience frosts (Geering *et al* 1995).

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).

Larval screw-worm flies are unlikely to persist long on carcases or skins, as they rely on blood for nourishment. AFFA (2001) determined 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 (DPIE 1996) 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 cypermethirn plus chlorfenvinphos as alternatives.

Conclusions:

- **Screw-worm fly is an insect pest that can breed on a range of animal hosts.**
- **Australia's response plan for a screw worm fly incursion does not include the slaughter of livestock or destruction of animal products.**
- **To prevent transportation of the fly to new regions, and to minimise breeding grounds for the fly, disinsection of faeces on plant and equipment may be necessary. Unprocessed hides being exported from a control area may also require treatment.**
- **Meat and milk pose a negligible transmission risk. No special disposal precautions are warranted.**

References:

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

DPIE (Department of Primary Industries and Energy) 1996, *Australian Veterinary Emergency Plan, AUSVETPLAN Edition 2.0, Disease Strategy Screw-worm fly,* DPIE, Canberra.

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.

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.

Braula fly

Agent:
Braula coeca

Agent type:
Fly

Persistence and inactivation:
Braula coeca is a dipterous fly and commensal of honey bees. Larvae pupate in tunnels below cappings, and on emergence, the adult flies attach to adult worker bees, queen bees and sometimes drones. DPIE (1996) states that *B. coeca* is not known to survive in the absence of adult bees, although it will survive in a colony without brood. No references to persistence off the host were found during this review.

B. coeca is spread by swarming or drifting colonies and by the transfer of colonies or hive components (DPIE 1996).

Kulincevic *et al* (1991) reported that fluvalinate as an aerosol or fumigant killed significant numbers of Braula but that amitraz was not effective.

Conclusions:

- **AUSVETPLAN stipulates the destruction of infected hives by either burning or treatment with an insecticide or solvent followed by burial.**
- **Given the low unit value of bees and their products and the relative ease of disposal, there is no obvious reason to consider lower cost, higher risk disposal methods.**

References:
DPIE (Department of Primary Industries and Energy) 1996, *Australian Veterinary Emergency Plan, AUSVETPLAN Edition 2.0, Disease Strategy Bee diseases*, DPIE, Canberra.

Kulincevic, J.M., Rinderer, T.E., & Mladjan, V.J. 1991, 'Effects of fluvalinate and amitraz on bee lice (*Braula coeca* Nitzsch) in honey bee (*Apis mellifera* L) colonies in Yugoslavia', *Apidologie*, vol. 22, pp. 43-47.

Small hive beetle

Agent:
Aethina tumida

Agent type:
Beetle

Persistence and inactivation:
Limited research exists on small hive beetle, as it has only been described as a pathogen of honey bee colonies in the Western Hemisphere since 1998. Larvae of *A. tumida* migrate from combs to pupate in surrounding soil. Studies in the US found beetle larvae, pupae and adults at a depth of 1-20cm in soil around hive entrances. Eighty percent of the beetles were within 30cm, and none were further than 180cm from the hive (Pettis and Shimanuki 2000). Development from egg to adult takes 38-81 days (Lundie 1940). Adults survived for up to five days without food or water in moderate summer temperatures (Pettis and Shimanuki 2000), but could survive much longer if provided food (e.g. greater than 60 days reported by Lundie (1940) for beetles fed honey and pollen). Elzen *et al* (1999) observed small hive beetle mortalities as high as 90.2% using 10% coumaphos in plastic strips.

Conclusions:

- **Small hive beetle would be more difficult to eradicate than other bee pests because of its capacity to survive free of the colony. However, the destruction of both hives and feral bee colonies would be an eradication option for Australia in the case of an isolated incursion.**
- **The preferred methods for destroying infected hives are either burning or treatment with an insecticide or solvent followed by burial.**
- **Given the low unit value of bees and their products and the relative ease of disposal, there is no obvious reason to consider lower cost, higher risk disposal methods.**

References:
DPIE (Department of Primary Industries and Energy) 1996, *Australian Veterinary Emergency Plan, AUSVETPLAN Edition 2.0, Disease Strategy Bee diseases*, DPIE, Canberra.

Elzen, P.J., Baxter J.R., Westervelt, D., Randall, C., Delaplane, K.S., Cutts, L., & Wilson, W.T. 1999, 'Field control and biology studies of a new pest species, *Aethina tumida* Murray (Coleoptera, Nitidulidae), attacking European honey bees in the Western hemisphere', *Apidologie*, vol. 30, pp. 361-366.

Lundie, A.E. 1940, *The small hive beetle,* Aethina tumida, *Sci Bull 220,* Department of Agriculture and Forestry, Union of South Africa, 30pp, cited in Elzen *et al* 1999.

Pettis, J.S., & Shimanuki, H. 2000, 'Observations on the small hive beetle, *Aethina tumida* Murray, in the United States', *Am Bee J*, vol. 140, pp 152-155.

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Trichinellosis

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.

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.

According to Urquhart *et al* (1987), *Trichinella* maintain infectivity in decomposing carcases 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^{o}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^{o}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.

Smoking, drying or curing pork does not necessarily inactivate *Trichinella* larvae (Urquhart *et al* 1987).

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

For irradiation, the ICT recommends 0.3 kGy (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.

Conclusions:

- ***T. spiralis* larvae encapsulated in muscle cells remain viable well beyond the life of the animal host.**
- **Transmission occurs with the ingestion of muscle tissue containing *T. spiralis* cysts or the ingestion of fresh faeces from an infected animal.**
- **Fresh carcases must be disposed of in a manner that precludes scavenging.**
- **Infected meat poses a disease transmission and human health risk. *T. spiralis* cysts can be destroyed by freezing, heating and/or irradiation, but this must be done under controlled conditions to ensure inactivation of the parasite.**
- **Skins and milk pose a negligible transmission risk. No special disposal precautions are warranted.**

References:

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Appendix 1

Example search strategy

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 stategy:
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