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Risk assessment of pathogens of bovine blood

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Executive summary

A pilot scale ion-exchange, continuous chromatographic separation process (CSEP) has been developed for the fractionation of bovine plasma proteins. These proteins have the potential to be used in food preparations, and also to be further processed into nutraceutical or pharmaceutical products. In order to implement the technology at a commercial level, it is important to assess the risk of zoonotic disease being transmitted to operators of the technology, and the risks related to zoonotic agents being concentrated in waste streams or in the final product. This project aims to qualitatively assess the zoonotic risks using standard Occupational Health and Safety (OH&S) risk assessment methodology.

OH&S risks explored include zoonotic agents potentially carried in or transmitted through bovine blood or plasma. The pathogens of concern were identified through review of relevant literature and national surveillance reports, including those provided by Meat and Livestock Australia (MLA). For the identified hazards, the routes and vehicles of transmission are described in the report, and the effect of the hurdles imposed by the CSEP procedure on the potential for transmission outlined. Commonly recognized hazards, such as those associated with manual lifting are not included.

A number of potential zoonoses are not present in Australia, being the subject of national surveillance and control measures. A further subset, particularly the nematode or trematode parasites, and skin infections such as ringworm, would not be transmissible through blood. Bacterial agents in blood would be the result of contamination during collection and handling, so hygienic procedures at this stage will minimise the risk, and furthermore, the size of the organisms should lead to their removal from plasma when the blood is centrifuged. Q fever and babesiosis are caused by intracellular organisms, and again should be concentrated in the sedimented cellular fraction following centrifugation. Both organisms are present in Australia at a low level, babesiosis being associated with Northern areas of Australia, where it is transmitted between cattle via the cattle tick *Boophilus microplus*, and Q fever being estimated to be present in approximately 12% of cattle. Q fever illness in humans affects individuals exposed to the organism in contaminated dust, particularly from dairy farms, or those exposed to uterine secretions through assisting in calving, or processing the genitalia of cattle during slaughter.

This Risk Assessment provides a guide to the zoonotic hazards that may be encountered when processing bovine blood for the production of bioactive agents. It is vital that individual processors carry out their own Risk Assessment, particular to the specific conditions pertaining to their operation, as this guideline document makes certain assumptions, particularly with regard to the susceptibility of the workers and the presence of zoonotic agents in cattle.

When bovine blood is harvested for human consumption and processed for the production of bioactive agents, the risk to workers presented by zoonotic agents is greatest in the early stages of the process, and at a level similar to that encountered in routine beef production. The centrifugation step itself results in a reduction of risk associated with the plasma fraction, but concentrates any zoonotic agents present into the cell fraction. Handling of the cell fraction, for further processing or for disposal, thus becomes a greater risk operation, and additional precautions such as personal protective equipment should be considered.

The likelihood of zoonotic agents being transmitted through the process and into the product is very low, as most, if not all, should be removed at centrifugation.

Contents

1 Introduction

Bovine blood collected at the abattoir has many potential uses including the production of nutraceutical and pharmaceutical products. Between 10 and 15 kg of blood can be collected from each slaughterweight bovine, a yield of some 2.5-6% of the animal's liveweight. Blood is a fluid consisting of approximately 80% water and 20% solids. It consists of a translucent yellow fluid, plasma, in which are suspended red and white blood cells, platelets and proteins. When blood is collected, the platelets and blood cells rapidly coagulate forming a clot, releasing serum. The clot binds some of the blood proteins, so to prevent clotting, 3 g/l citrate is mixed into the blood immediately after collection. The blood cells can be removed from the citrated blood by centrifugation, and the plasma harvested for separation into its protein fractions.

Potential food applications for plasma protein fractions include bakery, processed meat products and health beverages based upon the functional properties and competitive cost of these ingredients. Plasma protein fractions have been reported to have excellent heat set gelation properties forming bland-tasting white gels. This functional property could be exploited as a fat substitute in smallgoods and other food products. The plasma proteins have also been reported to have emulsifying and excellent water holding capacity over a wide pH range. They can be used as a replacement for soy protein isolate or non-fat dried milk in many processed foods including meat-based products. Plasma-derived fractions could also provide relatively pure protein sources for the manufacture of high value pharmaceutical, veterinary or diagnostic products.

Blood protein fractions, while having clear potential as new and novel food ingredients in their own right, also have potential as the feedstock for 'second generation' products, such as bioactive peptides following secondary processing of the primary fractions. These peptides could provide the Australian meat processing industry with an entry point into the lucrative global functional foods and nutraceutical markets. Potential applications of bioactive peptides include control of hypertension/blood pressure through inhibition of a key enzyme (angiotensin converting enzyme), analgesic and sedative effects on the nervous system, and control of angiogenesis to name just a few. The bioactive peptides could be incorporated into processed meats, potentially delivering additional health benefits. Similarly, antibacterial and antifungal properties derived from hydrolysed plasma proteins could also have applications in the meat and general food industries.

The bioactive components of plasma, such as bovine serum albumin (BSA), transferrin, Immunoglobulin-G and protease inhibitors, can be separated by ion-exchange chromatography, and a pilot scale continuous chromatographic separation (CSEP) process has been developed at Food Science Australia (FSA), Werribee. This CSEP technology overcomes many of the shortcomings associated with traditional chromatography, as well as meeting the need for a cost-effective commercial process. The CSEP technology incorporates all the steps of traditional chromatography, such as washing, loading and elution, but in a continuous manner. This approach also simplifies upstream and downstream processes and allows them to operate continuously. As a result of the continuous nature of the CSEP technology and the simple process control requirements, the operational cost of this approach is low in comparison to traditional chromatographic systems. The CSEP technology is also directly scaleable and is capable of processing fluids over a very wide range of flow rates and hydraulic characteristics.

When any new process is implemented in a commercial situation, it is vital that any Occupational Health and Safety (OH&S) issues are identified and appropriately controlled. This project aims to identify OH&S risks arising during the normal operation of the process and during routine cleaning and maintenance, and to carry out a Qualitative Risk Assessment on these. From this Risk Assessment, guidelines for installation, operation and maintenance can be developed.

2 Project aims

- Identification of OH&S risks associated with chromatographic separation of bioactive agents from bovine blood or plasma, including waste handling and disposal.
- Perform OH&S Risk Assessment
- Develop guidelines for the overall process including installation, operation and maintenance of the equipment under normal commercial circumstances.

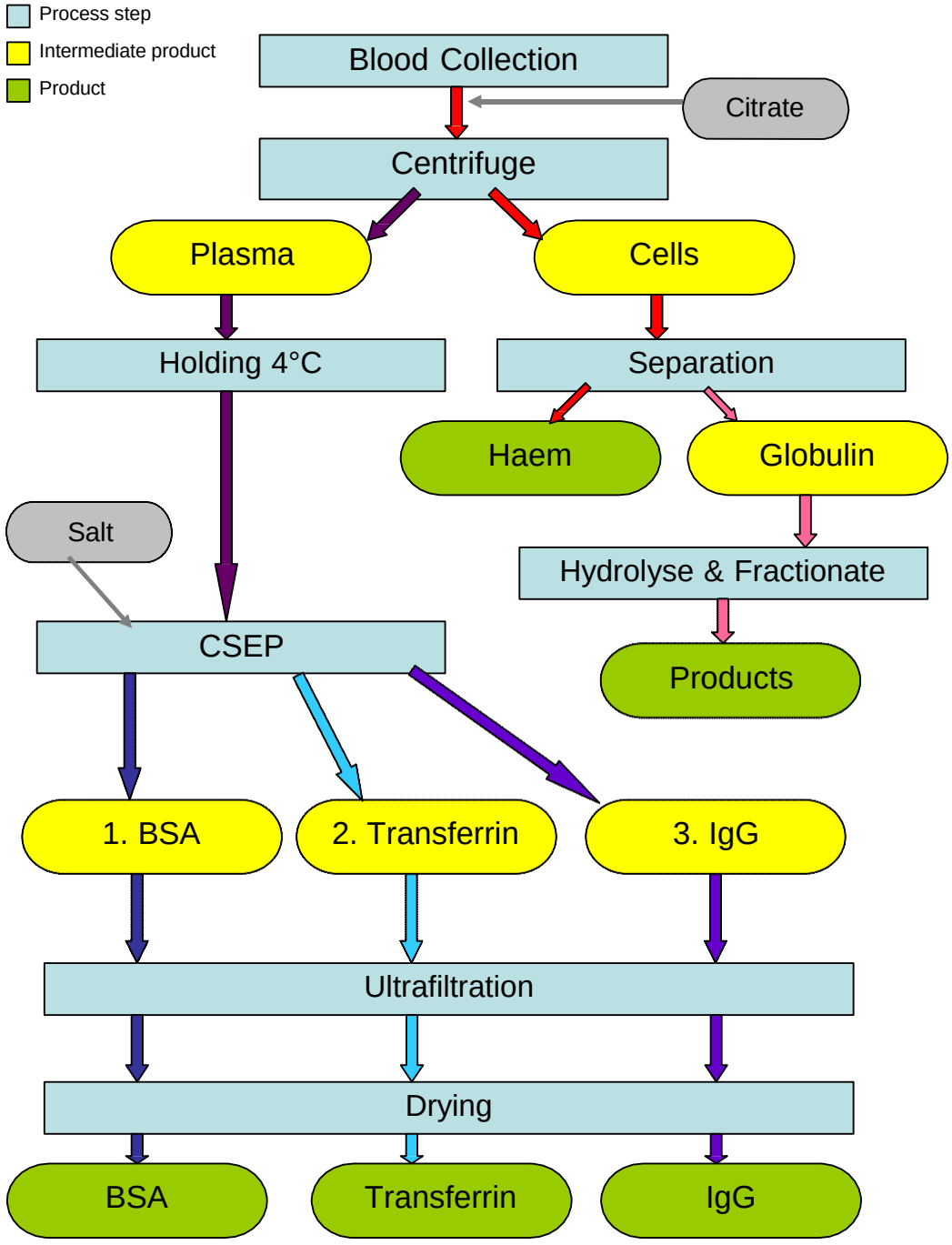
3 Methods

3.1 Description of Fractionation procedure

Blood is collected hygienically from bovine animals at the sticking point during normal slaughter. It is immediately citrated and centrifuged using a commercial blood separator, and the plasma supernatant separated from the cell component. The cell component could be further processed, but this procedure is outside the scope of the current project.

The plasma is chilled and held at 4°C or lower prior to fractionation via the CSEP process. CSEP is an automatic enclosed system where plasma proteins are bound to a resin according to ionic charge, and then eluted in sequence by increasing the salt concentration, up to 0.5M NaCl, in the fluid flushed through the column. Three fractions are eluted: Immunoglobulin-G (IgG), transferrin (TF) and BSA. These are then concentrated and desalted by ultrafiltration through a 10 kDa filter. A flowchart of the process is shown in Figure 1.

Figure 1: Processing blood for the production of bioactive agents



3.2 Identification of zoonotic hazards

Zoonotic agents associated with cattle were identified using a variety of sources, including textbooks, previous MLA reports and web-based material. The presence of these agents in the Australian cattle population was ascertained from the Animal Health Australia website, and the occurrence of the associated illnesses in the Australian human population from the Australian Government Department of Health and Ageing website. The reported routes of transmission and pathogenesis of each illness was considered, with a view to assessing the likelihood of each agent being present in bovine blood at collection, and the physical and persistence characteristics of each agent taken into account.

3.3 Qualitative Risk Assessment

A matrix method of assessing risk was applied to the identified zoonotic pathogens, in order to carry out a qualitative risk assessment. Recognized hazards common to all meatworks operations, such as those associated with manual lifting are not included.

Many disease agents have a more serious outcome in susceptible populations, for example infants, the elderly, immunocompromised individuals or pregnant women. For the purpose of this qualitative risk assessment it was assumed that the exposed individuals, those that would be involved in operation of the blood collection and fractionation process, are healthy immune-competent adults, and not belonging to a sub-population of increased susceptibility.
Risk Estimation was carried out using a Risk Matrix approach as described in Australian Standard 3931:1998, to give a qualitative assessment of the relative risks relating to each zoonosis. For each condition, the Inherent Risk was estimated using the Risk Matrix shown in Table 1. The definitions of each consequence and likelihood descriptor are shown in Tables 2 and 3 respectively.

Table 1: Inherent risk matrix

			Consequence		
Likelihood	**Insignificant**	**Minor**	**Moderate**	**Major**	**Catastrophic**
Almost Certain	**M**	**M**	**H**	**H**	**H**
Likely	**M**	**M**	**M**	**H**	**H**
Possible	**L**	**M**	**M**	**H**	**H**
Unlikely	**L**	**L**	**M**	**M**	**H**
Rare	**L**	**L**	**M**	**M**	**H**

L – Low Risk; M – Moderate Risk; H – High Risk

Table 2: Definitions of consequence descriptors

Descriptor	**Consequence Description**
Insignificant	No illness in affected person; asymptomatic
Minor	Illness can occur; some people would have symptoms; minor symptoms, not requiring medical attention; short absence from work
Moderate	Illness usually occurs as a result of exposure; many people would have symptoms; temporary absence from work; requires medical attention
Major	Exposure to agent always results in illness; illness expected to occur; most people would have symptoms; prolonged absence from work; moderate to severe health effects; may be fatal
Catastrophic	Results in multiple fatalities

Table 3: Definitions of likelihood descriptors

Descriptor	Likelihood of Exposure
Almost certain	Activity always leads to exposure
Likely	Exposure is common
Possible	Exposure to agent can occur and would not be surprising
Unlikely	Exposure may occur, but would not be considered to be likely
Rare	Exposure is possible but very unlikely

Each stage in the blood fractionation process, from collection to spray drying, was then considered separately with a view to assessing the controls incorporated at each stage, and estimating the residual risk posed by the individual zoonotic agents at that stage in the process. The controls in place were rated Poor, Reasonable or Very Good in accordance with the definitions shown in table 4, and the residual risk estimated using the matrix shown in table 5, plotting inherent risk against control rating.

Table 4: Definitions of control ratings

Rating	Definition
Very Good	Best practice controls; emphasis is on elimination or reduction of risk
Reasonable	Controls are in place but risk could be further minimised
Poor	No controls in place

Table 5: Residual risk matrix

	Control rating		
Inherent Risk	**Very Good**	**Reasonable**	**Poor**
High	**M**	**H**	**H**
Moderate	**L**	**M**	**H**
Low	**L**	**L**	**M**

L – Low Risk; M – Moderate Risk; H – High Risk

4 Findings

4.1 Zoonotic illnesses associated with cattle

A total of 39 potential zoonoses associated with cattle were identified, 13 of which were reported to be not present in Australia. National surveillance confirms the presence of 14 of these zoonoses, and the additional 12 zoonoses were listed as likely to be present in Australia, but are not the subject of national surveillance programs. The 39 zoonoses are presented in Tables 6a-d, with a brief comment on the important aspects of each agent with regard to their presence in Australia and likely transmission via bovine blood.

Table 6a: Zoonotic illnesses associated with cattle

Present in Australia

1. OIE-listed*

Disease	Comment
Anthrax	Limited distribution in Australia, occurs sporadically. Not present in blood until terminal bacteraemia develops
Babesiosis	Present in northern and eastern parts of Australia. Organism exists in red cells. May be released into plasma
Cysticercosis	Oncospheres in blood are not the infective stage
Hydatid disease	Oncospheres may be present in blood, but most infections are faecal-oral from dog faeces
Leptospirosis	May be present in blood during initial bacteraemia and fever. Infections associated with ingestion via urine
MAP/Johne's disease	Present in south eastern states of Australia. Possible association with Crohn's disease, organism may be present in bovine faeces
Q fever	Obligate intracellular parasite, present in alveolar macrophages
West Nile Fever (Kunjin, Akabane)	Mosquito borne flavivirus. Occasionally reported in Australia, usually associated with birds

* Diseases listed by the Office International des Epizooties (OIE) as being of major importance to human health, animal health or international trade. OIE list A diseases are internationally notifiable, list B may be notifiable at a National level.

Table 6b: Zoonotic illnesses associated with cattle

Present in Australia

2. Non – OIE-listed

Disease	Comment
Clostridial diseases	Faecal-oral spread. Clinical cases in animals obvious
Campylobacteriosis	Carried asymptomatically in faeces, not present in blood*
E. coli HC/HUS	Carried asymptomatically in faeces, not present in blood*
Salmonellosis	Carried asymptomatically in faeces, not present in blood. Clinical cases in animal obvious*
Listeriosis	Present in faeces, urine and milk. Present in blood during clinical phase, animal is fevered*
Pasteurellosis (Shipping fever)	Usually peracute in the animal, so clinical cases obvious

Providing blood is removed aseptically prior to carcass opening, there should be little risk from these organisms

Table 6c: Zoonotic illnesses associated with cattle

Likely to be present – Not covered by surveillance programs

Coenurosis	Associated with sheep. Oncospheres may be present in blood, but most infections are faecal-oral from dog faeces
Cowpox	Direct skin contact
Corynebacteriosis	Caseous lymphadenitis is associated with sheep
Cryptosporidiosis	Faecal-oral transmission
Liver fluke	Requires intermediate host
Pseudocowpox	Requires direct skin contact
Ringworm	Requires direct skin contact
Sarcosporidiosis	Humans are definitive host, requires intermediate host, ingestion via meat
Schistosomiasis	Schistosomula migrate via the bloodstream. Probably not infective to humans. Requires intermediate host
Scabies	Requires direct skin contact
Thelaziasis	Not present in blood – roundworm transmitted by flies
Yersiniosis	Faecal-oral transmission, may be present in blood, but with fever

Table 6d: Zoonotic illnesses associated with cattle

Not present in Australia – covered by surveillance programs

Disease	**Comment**
Bovine Brucellosis	Eradicated 1989
Bovine TB	Eradicated 1997, last occurrence 2002
BSE	Never occurred in Australia
Congo fever	Never occurred in Australia
Foot and mouth disease	Last outbreak 1872, requires high exposure to cause illness in humans
Ganjam/Dugbe virus	Never occurred in Australia. Associated with sheep.
Haemorrhagic septicaemia	Caused by *Pasteurella multocida* strains 6b, 6e. Never occurred in Australia
Rabies	Last occurred 1867
Rift Valley Fever	Never occurred in Australia
Russian tick-borne encephalitis	Never occurred in Australia
Trypanosomiasis (Chaga's disease)	Never occurred in Australia
Vesicular Stomatitis	Never occurred in Australia
Wesselsbron Disease	Never occurred in Australia

A number of the zoonotic illnesses can be discounted for the purposes of this risk profile due to the fact that they either do not occur in Australia, or because transmission is through direct contact with the infected animal or its surroundings (Table 7), as the organism is present only on the skin. Similarly, the nematodes and trematodes can be discounted, as their lifecycle requires specific conditions of transmission in order to cause illness.

Table 7: Zoonotic agents unlikely to be present in bovine blood

Other zoonoses	
Cysticercosis **Hydatidosis** **Coenurosis** **Liver fluke** **Schistosomiasis**	Clinical cases blocked at ante-mortem and post-mortem inspection. Pre-clinical stages unlikely to be infective
Thelaziasis	Insect-borne roundworm that does not enter the circulatory system
Cowpox **Pseudocowpox** **Ringworm** **Scabies**	Superficial infections that do not enter the circulatory system. Require direct contact with the affected animal or the animal environment

4.2 Illnesses caused by bacterial agents

Bacteria are unicellular organisms that range from 0.5 to 6 µm in size. Many of the pathogens identified are carried in the faeces of healthy cattle, and thus would only be present in whole blood as a result of faecal contamination during collection or handling. Others, those that truly cause disease in cattle, would only enter the bloodstream in the terminal stages of illness, when the disease would be apparent at ante-mortem or post-mortem inspection, and thus the blood should be rejected as unfit for human consumption. Blood separation by centrifugation aims to remove solids that are larger than 0.2 µm in size from the plasma, so any bacteria present should be concentrated in the cell fraction at this stage.

4.2.1 Campylobacteriosis

The *Campylobacter* species associated with human illness are *Camp. jejuni*, *Camp. coli*, and *Camp. fetus*. They are Gram negative slender, spiral and curving rods, 0.2-0.5 µm wide and 1.5-5 µm long. *Campylobacter* are microaerophilic and sensitive to drying, UV light, pH<4.5 and heat, being inactivated at temperatures above 48°C. They are normal inhabitants of the bovine intestine (Fricker and Park, 1989), and are shed in the faeces. Feedlot cattle seem to be more likely to carry *Campylobacter* than grazing cattle (Giacoboni *et al*, 1993), and 12.5% of Australian cattle at slaughter have been found to shed the organism in the faeces (Grau 1988).

Campylobacteriosis is probably one of the most common foodborne illnesses worldwide, with around 100-125 reported cases per 100,000 people per annum in Australia. There is probably an overall case rate of 30-100 times this, due to the vast number of cases not investigated to the confirmatory level. It is transmitted by the faecal-oral route, via contaminated food or water, and many cases are attributed to poultry.

There is an incubation period of 1-10 days, followed by acute onset fever with severe abdominal pains and watery diarrhoea. The diarrhoea usually stops after 5-10 days, but some cases have more persistent diarrhoea for up to 4 weeks. There are rare cases of arthritis or neurological illness following *Campylobacter* enteritis, with the inflammatory demyelinating polyradiculomyopathy Guillain-Barre Syndrome occurring in about 0.1% of clinical cases. Illness in immunocompromised patients is more severe.

Campylobacter species are carried in the faeces and would only be present in bovine blood through contamination. They should be removed during centrifugation.

4.2.2 Salmonellosis

There are over 2400 serotypes of *Salmonella*, a number of which cause illness in a number of animal species. Some pathogenic salmonellae are very host-specific, while others are true zoonoses, causing illness in more than one species, including humans. Those which are the true zoonoses cause clinically evident signs in animals, so infected animals should be screened out at ante-mortem and post-mortem inspection, and the blood rejected as unfit for human consumption. Some serotypes that are pathogenic to humans may be carried asymptomatically in the intestine of cattle and excreted in the faeces. Salmonellae are Gram negative rods, 0.5x3 µm in size. Most strains are inactivated by pasteurisation, which is a heat treatment. Pasteurisation may be carried out by the holding, or vat, method which is no less than 62.8°C for 30 minutes, or by the high temperature short time (HTST) method which is no less than 71.2°C for 15 seconds.

Humans are affected by ingestion of the organism in contaminated food, and the severity of illness depends on the number of salmonellae ingested. There is an incubation period of 6-72 hours, followed by watery diarrhoea for up to 10 days with low grade fever and abdominal pain. Most cases are self-limiting, but the elderly, immunocompromised or very young may die as a result of dehydration. Reactive arthritis is a rare sequel.

There are some 35-40 reported cases of salmonellosis per 100,000 in Australia per annum, and this again is likely to be a gross underestimate of the true burden of disease. Surveillance studies at abattoirs have indicated that up to 100% of cattle may carry *Salmonella* spp in the faeces, but this includes non-pathogenic strains as well as pathogenic strains (FSA 2002; Fegan *et al*, 2005).

Salmonellae are carried in the faeces and would only be present in bovine blood through contamination. They should be removed at centrifugation.

4.2.3 Shigatoxigenic E. coli (STEC)

Escherichia coli are Gram negative rods, typically 1.1-1.5 µm, wide by 2-6 µm long, and are the most common organism in the intestinal tract of all species. Many strains are non-pathogenic, but others cause a range of syndromes in humans and animals (Cole *et al*, 1999). Recently, a group of *E. coli* have emerged that produce a Shiga toxin, which is responsible for severe illness in humans. These STEC are associated with Haemorrhagic Colitis, and Haemolytic Uraemic Syndrome (HUS), the primary cause of acute renal failure in children under the age of ten. The infective dose of STEC seems to be quite low (Desmarchelier and Grau 1997), and there are around 0.2-0.4 reported cases per 100,000 per annum in Australia.

E. coli are found in many species at slaughter, but STEC are less commonly isolated. Studies in Australia have indicated prevalences between 0.2 and 32% (Cobbold and Desmarchelier, 2000; Hornitzky *et al*, 2001; Hallaran and Sumner, 2001; Midgley and Desmarchelier, 2001; Fegan *et al*, 2004; Fegan *et al*, 2005), and as detection methods improve, greater prevalences are being found. For example one study determining the presence of STEC *stx* genes in bovine faeces found within-lot prevalence to vary between 4% and 68%, possibly due to intermittent shedding (Midgley *et al*, 1999), or between 12 and 98% (FSA 1997).

STEC are carried in the faeces and would only be present in bovine blood through contamination. They should be removed at centrifugation.

4.2.4 Listeriosis

Listeria monocytogenes is a Gram positive nonsporeforming rod, diameter 0.5 µm, length 0.5-2 µm. It is widely distributed in animals, birds and soil, and is excreted in faeces. *Listeria* is important in food processing because it grows at refrigeration temperatures (<4°C). Pasteurisation readily inactivates *Listeria*. In the host, it is intracellular, and is transmitted from cell to cell using host cell proteins. *Listeria* is phagocytosed by the host cell, and escapes from the vacuole into the call cytoplasm. There, it multiplies, and utilises the actin filaments to migrate to the cell surface, where it is extruded in a pseudopod-like structure for phagocytosis by a second eukaryotic cell.

Listeria is a true zoonosis, and can cause encephalitis, septicaemia and abortion in cattle, but a number of cattle are symptomless carriers. There is little data on the carriage of *L. monocytogenes* in Australian cattle, but an estimate of 75% has been made (MLA, 2003), based on UK data (Nicholson *et al*, 1999). In humans, OzFoodNet recorded 66 cases of listeriosis in 2004, equating to approximately 0.3 per 100,000 head (OzFoodNet, 2006). Listeriosis is generally a mild illness with fever and ‘flu-like symptoms, but in susceptible groups (pregnant women, neonates, foetuses, the elderly and immunocompromised) the illness can be very severe, including septicaemia, meningitis/meningoencephalitis and encephalitis. Pregnant women may abort. About 20% of clinical cases will die.

Listeria is carried in the faeces and would only be present in bovine blood through contamination. Animals that are clinically infected should not be processed for human consumption. If present, the bacterium should be removed at centrifugation.

4.2.5 Clostridial diseases

The clostridia are responsible for a number of conditions in a variety of species. Botulism, tetanus, blackleg and gas gangrene are caused by members of this bacterial species, as well as an enterotoxaemia leading to enteritis. The organisms are Gram positive sporeforming anaerobes, that require the absence of oxygen to grow and multiply. Spores of many clostridial species are ubiquitous and highly resistant to heat, desiccation and other environmental factors.

Botulism is associated with growth of *Clostridium botulinum* in foods. While it grows it produces a neurotoxin, and the toxin, when ingested, causes flaccid paralysis, which may be fatal. Tetanus is associated with toxin produced by *Cl. tetani* when that organism multiplies in skin, producing a neurotoxin that causes tonic paresis. Gas gangrene and blackleg are associated also with wound contamination, with a number of different clostridia, most notably *Cl. perfringens*.

Cl. perfringens can also cause food poisoning following the ingestion of large numbers of vegetative cells from foods held for prolonged periods at 40 to 50°C, where cell growth can be extremely rapid. Enterotoxin is produced during sporulation of the cells in the human gut, resulting in illness. C. perfringens spores are ubiquitous in soil, and are excreted in animal faeces. The illness manifests as acute abdominal pain and diarrhoea lasting for about a day.

Clostridium spores are present in soil and carried in the faeces and would only be present in bovine blood through contamination. They should be removed at centrifugation as they are approximately 1 µm in diameter.

4.2.6 Leptospirosis

Leptospirosis tends to be an accidental infection related to occupational exposure to the organism in contaminated water. The organism, *Leptospira hardjo*, is excreted in the urine of cattle, and is contracted through the mucous membranes of the mouth, nose and eyes. There is an incubation period of 3-20 days, followed by a flu-like illness lasting 4-9 days. Meningitis may occur and last for up to 3 weeks. Intense headache is common and pharyngitis often occurs. Fatality rate is less than 1%. There are other leptospires, which cause a wide range of symptoms, such as *L. icterohaemorrhagiae*, which is carried by rats. It is the most pathogenic species, causing jaundice, haemolytic anaemia, meningitis, pneumonia and nephritis. Mortality from *L. icterohaemorrhagiae* can be up to 20%. There are approximately 0.6 cases of human leptospirosis per 100,000 per year in Australia.

L. hardjo is the strain associated with cattle, and is present in Australia. It is excreted in the urine and would only be present in bovine blood through contamination. It should be removed at centrifugation.

4.2.7 Bovine TB

Bovine TB is caused by *Mycobacterium bovis*, a slender bacillus 1-4 µm in length. It is a facultative intracellular pathogen, so lives inside host cells. Human tuberculosis is generally caused by the related organism *Mycobacterium tuberculosis*. In cattle the illness is primarily an airborne infection. *M. bovis* are phagocytosed by alveolar macrophages, in which they begin multiplying. The organism is spread round the body via the blood when tubercles rupture. The illness in cattle is subclinical for long periods, but eventually leads to signs of chronic bronchopneumonia, progressive respiratory distress and death. Indurative mastitis may occur. The organism is excreted in milk, urine, faeces and sputum. It has been eradicated from Australia, and the country was declared officially free in 1997. Sporadic cases can still be found, the most recent of which was 2002.

Human infection with *M. bovis* can occur through ingestion of the organism, most often via raw or improperly pasteurised milk. There may be fever, abdominal discomfort and weight loss. Inhalational infection can occur in individuals handling infected tissue and leads to more severe symptoms. Many infections are asymptomatic, but clinical illness is usually chronic, with

symptoms of cough, fever, fatigue, inappetance, weight loss, chest pain and haemoptysis (coughing blood).

Bovine TB is very unlikely to be present in cattle processed for human consumption in Australia. Post mortem meat inspection focuses on the detection of cases of bovine TB, and carcasses should be rejected as unfit for human consumption. The organism should also be removed with the cellular component during centrifugation.

4.2.8 Crohn's disease (Paratuberculosis)

Mycobacterium avium paratuberculosis (MAP) is associated with Johne's disease in cattle, and may be associated with Crohn's disease in humans. It is an obligate parasitic pathogen; all replication occurs inside infected host macrophages.

In cattle, it is acquired by ingestion when the animal is quite young, and there is generally a long incubation period. It crosses the intestinal wall and enters the macrophages, which transport it to local lymph nodes. Clinical signs are of chronic enteritis, diarrhoea, lymphadenpopathy, emaciation and alopecia. It is a protein-losing enteropathy, and ultimate hypoalbuminaemia leads to an inability to retain fluid in the circulatory system, leading to dependant oedema and bottle-jaw. The organism enters the blood stream at the terminal stages, but at this point the animal should be rejected on ante-mortem inspection as unfit for consumption.

MAP is common in developed livestock production systems worldwide, especially in dairy populations. In Australia, it is restricted to south eastern Australia, and the herd prevalence is low. Approximately 10% of dairy herds and 0.09% of beef herds are positive, and within infected herds, animal prevelance is below 10% (MLA, 2003).

MAP has been isolated from humans with Crohn's disease. Because of some clinical and pathological similarities between Crohn's disease in humans and Johne's disease in animals, it is believed that MAP may also cause Crohn's desease (Rubery, 2001).

MAP is carried in the faeces and would only be present in bovine blood through contamination. It should be removed at centrifugation.

4.2.9 Pasteurellosis

Pasteurella multocida is associated with respiratory illness in a number of species. There is a notifiable disease, Haemorrhagic Septicaemia, associated with particular strains of the organism, but neither this disease nor the associated strains have been identified in Australia.

Clinical cases of pasteurellosis in animals are often peracute, and the animal is found dead, for example shipping fever in sheep. Clinically ill animals should not be processed for human consumption. Human illness is associated with inhalation of the organism, and can result in respiratory problems. Most cases occur in immunocompromised individuals

P. multocida is unlikely to be present in bovine blood, unless through contamination. The organism should be removed with the cellular component when the whole blood was centrifuged.

4.2.10 Yersiniosis

Yersinia enterocolitica has been isolated from cattle, but currently only isolates from pigs correlate well with isolates from humans. The organism is a Gram negative non-sporeforming coccobacillus, 0.5-0.8 µm in diameter and 1-3.5 µm in length. It is distributed widely in the environment. Animals are symptomless carriers, and transmission to humans is via the faecal-oral route. There are 3-7 days' incubation, followed by acute onset fever, with abdominal pains and watery diarrhoea that persists for 1-3 weeks. OzFoodNet reported 108 cases of yersiniosis in Australia in 2004, equating to 1.3 per 100,000 head of population. Most of these were in Queensland. Yersiniosis is not notifiable in Australia, although most states still record its presence (OzFoodNet, 2006).

It is confined to the intestine of cattle, so should only be present in blood following faecal contamination. It should be removed by centrifugation.

4.2.11 Corynebacteriosis

This illness is more likely to be associated with sheep than cattle. *Corynebacterium pseudotuberculosis* causes Caseous Lymphadenitis in sheep, and can cause skin infections with swollen lymph nodes in humans. Humans are infected through skin wounds. It is unlikely to be found in bovine blood and should be removed during centrifugation.

4.2.12 Anthrax

Anthrax is a notifiable disease caused by the organism *Bacillus anthracis*. The organism has limited distribution in Australia, sporadic cases in livestock being reported in New South Wales and Victoria. The illness is considered to be an occupational hazard of persons handling infected animals and their products. Spores of the organism can survive for up to 20 years in soils, hides, wool, feeds and fertilisers prepared from animals that die of anthrax. The illness in livestock is usually peracute, so the animal is found dead or dying. Clinically affected animals should not be processed for human consumption, and it is unlikely that they would even reach the abattoir. Cells and spores are relatively large (1.0-1.2 µm in diameter, 3-5 µm in length) and should be removed by centrifugation if present.

4.3 Illnesses caused by viral agents

Viruses are minute packages of genetic material that require host cells in which to multiply. There are a number of zoonotic viruses associated with cattle, the majority of which are not present in Australia, and are controlled through national surveillance and eradication programs (Table 1c). Cowpox and pseudocowpox may be present in the Australian cattle population, but are not reportable, so there is no data available on their incidence. In both cattle and in humans, these viruses result in mild self-limiting skin conditions. There are occasional reports of flaviviridae belonging to the West Nile Fever – Kunjin – Akabane group in Northern Queensland. These viruses are transmitted through mosquito bites, and the reservoir is usually considered to be wild birds rather than cattle. In cattle, West Nile Fever is usually subclinical, and in humans it results in a sudden onset fever, with headache, muscle pains and swollen lymph nodes. It is usually self-limiting, but recovery may be prolonged. It is unlikely that these viruses would be of concern in blood separation as firstly, they are intracellular, so will be removed with the blood cells during centrifugation, and secondly, they require to be inoculated into the victim through the bite of a mosquito.

4.4 Illnesses caused by other agents

4.4.1 Q Fever

Q fever is caused by the organism *Coxiella burnetii*, a pleomorphic Gram-variable rickettsia 0.2-0.7 µm in size. It is an obligate intracellular parasite, i.e. it cannot multiply outside host cells. In natural hosts it propagates primarily in the alveolar macrophages before disseminating. There are two cellular types, small cell variants (SCV) which are metabolically dormant, and large cell variants (LCV) which are metabolically active. It is suggested that SCVs are phagocytosed by eukaryotic host cells, where they differentiate into LCVs. There are also specific spore-like particles (SLP) which may be the form responsible for the organism's high resistance to harsh environmental conditions. The SLPs are excreted in urine and uterine secretions and can survive in soil for years. They can be extremely infective – a single cell may be sufficient to cause infection.

Humans are infected mainly through the inhalation of infected dust, although most infections are subclinical. There is a 2-6 week incubation period, and then there may be 'flu-like symptoms lasting for 7-10 days. Recovery can be prolonged, but most cases recover fully within a few

weeks. Up to 20% of clinical cases may result in chronic problems such as endocarditis or hepatitis, up to 20 years following the initial illness (Cole *et al*, 1999).

In livestock, transmission is probably through inhalation, but respiratory pathology and chronic conditions such as endocarditis or hepatitis do not seem to occur. There are usually no signs of illness, although abortion has been attributed to Q fever infection. The organism can persist in the udder and uterus for months. Handling of cattle during calving is a high-risk activity (Welsh *et al*, 1958).

Q fever is present in Australia, and the prevalence of carrier cattle has been estimated to be 0-25.1% most likely 11.78% (MLA 2003; Williams, 1991; Lang, 1990; Stoker *et al*, 1955). The organism lives inside the host cells, and is larger than 0.2 µm in size, so is likely to be removed with the cellular components during centrifugation.

4.4.2 Cryptosporidiosis

The organism, *Cryptosporidium parvum*, is a coccidion, a protozoan parasite. The infective stage, the oocyst, is 4-5 µm in size. The organism is highly resistant and will survive low temperature pasteurisation or freezing. The organism is transmitted through the faecal-oral route, and does not enter the blood stream. It is only likely to be present in blood that has been contaminated with faecal matter during collection or handling at the abattoir, and will be removed with the blood cells during centrifugation.

Cryptosporidiosis occurs worldwide, and is often associated with drinking water that has been contaminated with animal faeces or human sewage. Animals show no overt sign of illness, although young calves may develop diarrhoea (Cole *et al*, 1999). In humans, there is a 1-10 day incubation period, followed by mild mucoid diarrhoea for 1-2 weeks, with vomiting, fever, headache and abdominal pain. Most cases are self-limiting, but in susceptible individuals, such as the immunocompromised, the elderly, or very young it may be life-threatening (Teunis *et al*, 2002; ICMSF, 2002).

In Australia, it is considered that the prevalence of carrier cattle is 0-66.4%, most likely 1.1% (MLA, 2003; Scott *et al*, 1995).

4.4.3 Babesiosis

Babesiosis, or redwater fever in cattle, is caused by the intraerythrocytic (within red blood cells) protozoan *Babesia* species. There are two members of the species present in Australia, *B. bovis* and *B. bigemina*. *Babesia* are between 1 and 5 µm long, pyriform (flame-shaped), round or cigar-shaped. They divide asexually, within red blood cells, which eventually rupture, releasing the organisms into the plasma, from which they penetrate new red cells. Babesiosis is transmitted by ticks, and the organism undergoes developmental stages within the tick, so it is unlikely that direct infection can occur (Homer *et al*, 2000). The cattle tick *Boophilus microplus* is present in northern Australia and eastern Queensland, and bovine babesiosis occurs sporadically in these areas. In Queensland, cattle may be vaccinated against babesiosis.

In cattle, the classical signs of infection are fever, anaemia and haemoglobinuria (red urine due to free haemoglobin released from ruptured blood cells). The affected red blood cells may collect in the capillaries, leading to tissue damage, which, if it occurs in the brain, can cause incoordination and convulsions. If they recover, cattle develop immunity. In humans there is an incubation period of 1-12 months, followed by fever and lethargy. Destruction of blood cells leads to anaemia, jaundice, haemoglobinuria and kidney failure.

Babesiosis is unlikely to be of concern in plasma separation, as clinical cases in cattle will not pass ante-mortem inspection at the abattoir, centrifugation will remove the affected red cells or free piroplasms, and the tick is probably an essential stage in the transmission cycle.

4.4.4 Sarcosporidiosis

Sarcocystis hominis is an intracellular coccidion of which humans are the definitive host, and cattle the intermediate host. The sporocysts present in human faeces are infectious to cattle. When ingested, they penetrate the intestinal wall and travel as schizonts to the muscle. There, they enter the endothelial cells and develop into bradyzoite cysts, 0.5-5 mm in diameter. These are the infective stage for humans.

In cattle there are no signs of infection, but the cysts may be noticed as greenish discolouration in muscle at carcass breaking and cutting. Humans are infected by eating raw or undercooked beef containing the bradyzoite cysts. Infection is usually asymptomatic, but high doses can lead to nausea, abdominal pain and diarrhoea.

The infective stage is not present in bovine blood, and the schizonts are 2-8 µm in size, so should be removed by centrifugation.

4.4.5 Transmissible Spongiform Encephalopathy (TSE)

The cattle form of a Transmissible Spongiform Encephalopathy, BSE, has never occurred in Australia, but it is mentioned here due to its novel aetiology. The illness is believed to be caused by an abnormally folded Prion Protein (PrP), and transmission is assumed to result from the consumption of animal tissues containing a high level of the infectious prion. PrP has a molecular weight of 33-35 kDa. The normal Prion (PrP^{c}) is 38% α-helix and 19% β-pleated sheet, whereas the TSE Prion (PrP^{sc}) is 30% α-helix and 43% β-pleated sheet. PrP is species specific, and its structure is varied by the presence of differing numbers of salycilic acid chains. There are around 20 different abnormal PrP structures associated with BSE.

PrP is absorbed in the gastrointestinal tract, and reaches the central nervous system (CNS) possibly via the lymphatic system. It is likely that this involves diffusion up the spinal cord, and this determines the “incubation period”. When abnormal PrP reaches the brain, it contacts endogenous normal PrP and induces the conformational change into abnormal PrP.

BSE is associated epidemiologically with cases of New Variant Creuzfeldt-Jakob Disease (nvCJD), although no single case of nvCJD has been directly linked to the consumption of BSE-infected beef or related by-products. CJD is a rare disease that is found worldwide at a rate of approx 1-2 cases per million per year. Several cases of nvCJD occurred in farmers within 6 years of the first recorded BSE cases. The incubation period was short, and may be explained by an inhalation transmission, and dissemination via the olfactory lobe and nerve.

Research has not demonstrated TSE infectivity for blood (Doherr, 2002), although highly sensitive detection methods can detect PrP in serum from clinically affected cattle (Trieschmann *et al*, 2005). Clinical cases should not pass ante-mortem inspection. It is very unlikely that the abnormal PrP would be present in blood in the healthy live animal, as it travels via nervous tissue. Mechanical stunning, however, can lead to dissemination of CNS tissue via blood-borne emboli (Schmidt *et al*, 1999; Anil *et al*, 2002; Coore *et al*, 2005). At one point, there was a concern that the prion may be present in lymphoid cells, but the only lymphoid cell type which has stained positive for PrP is the non-circulating follicular dendritic cells. However, if free PrP was found to be present in the blood stream, it would pass into the plasma, and be present during separation by CSEP. CSEP involves ion-exchange chromatography, so binding and elution of the proteins is dependant upon their electric charge. The charge of PrP is variable, depending on the salycilic acid chains within the structure, so it is not possible at this stage to predict in which fraction the PrP would concentrate.

5 Qualitative risk assessment

A total of 16 zoonotic conditions associated with cattle were identified as being likely to be present in Australia (Table 8). Of these, cattle carrying the Anthrax agent, *Bacillus anthracis*, are very unlikely to be delivered to the abattoir, as the illness is usually peracute and the animal is found dead. For this reason, Anthrax has been excluded from the Risk Assessment, as have conditions that are known through surveillance to be not present in Australia.

Table 8: Zoonoses that are likely to be present in Australia

Zoonosis	Distribution	Animal product affected
Bovine babesiosis	NE Australia	Red blood cells
Q fever (*Coxiella burnettii*)	Countrywide, particularly QLD	Urine, uterine secretions, respiratory secretions, milk
Sarcosporidiosis	Not known	Muscle tissue
Campylobacteriosis	Countrywide	Faeces
Salmonellosis	Countrywide	Faeces
STEC	Countrywide	Faeces
Leptospirosis	Countrywide	Urine
Listeriosis	Countrywide	Faeces
Pasteurellosis	Countrywide	Respiratory secretions
Cryptosporidiosis	Countrywide	Faeces
MAP	SE Australia	Faeces, milk
Yersiniosis	Countrywide	Faeces
Corynebacteriosis	Countrywide	Skin
Anthrax	Sporadic outbreaks	All tissues
Clostridial Diseases	Countrywide	Faeces
Bovine Tuberculosis	Eradicated but occasional cases	Milk, respiratory secretions

The outcomes of the Risk assessment are presented in Tables 9-14. Each stage of the process is presented separately: Blood Collection; Centrifugation: CSEP Fractionation and Spray Drying. At each stage, the assumption is made that the previous stage has been carried out incorporating the recommended controls – thus at the Centrifugation Stage, the zoonotic agents are concentrated in the cell fraction and only the plasma progresses to Fractionation stage, so the likelihood of exposure to the agents is reduced at the Fractionation stage.

Table 9: Risks associated with Blood Collection stage								
No.	**Specific Hazard**	**Description of outcome of hazard realisation**	**Inherent Risk***			**Minimum Recommended Controls**	**Residual Risk#**	
			C	**L**	**IR**		**M**	**RR**
1	Babesiosis	Fever, lethargy, anaemia, jaundice, haemoglobinuria, kidney failure	MA	R	M	Ante-mortem and post-mortem inspection removes animals with clinical signs from processing Use of enclosed collection system to prevent operator exposure Basic hygiene training	VG	L
2	Q Fever	'Flu-like symptoms for 7-10 days. Possible prolonged convalescence Possible chronic complications: hepatitis or endocarditis	MA	P	H	Basic hygiene training	P	H
3	*Sarcosporidiosis*	Usually asymptomatic; possible nausea, abdominal pain and diarrhoea	MI	R	L	Use of enclosed collection system to prevent operator exposure Basic hygiene training	VG	L
4	Campylobacteriosis	Fever, abdominal pains, diarrhoea. Rare chronic sequelae	MO	P	M	Basic hygiene training	R	M
5	Salmonellosis	Mild fever, watery diarrhoea	MO	P	M	Ante-mortem and post-mortem inspection removes animals with clinical signs from processing Basic hygiene training	R	M
6	STEC	Mild to bloody diarrhoea for 7 days with severe abdominal pain Severe complications include HUS and TTP	MO	P	M	Basic hygiene training	R	M

No.	Specific Hazard	Description of outcome of hazard realisation	Inherent Risk*			Minimum Recommended Controls	Residual Risk#	
7	Leptospirosis	'Flu-like symptoms for 4-9 days with severe headache Meningitis may occur	MO	P	M	Ante-mortem and post-mortem inspection removes animals with clinical signs from processing Basic hygiene training	R	M
8	Listeriosis	Mild 'flu-like conditions Can lead to septicaemia and encephalitis	MO	P	M	Ante-mortem and post-mortem inspection removes animals with clinical signs from processing Basic hygiene training	R	M
9	Pasteurellosis	Can cause respiratory illness	MI	U	L	Ante-mortem and post-mortem inspection removes animals with clinical signs from processing Basic hygiene training	R	L
10	Cryptosporidiosis	Mild diarrhoea with fever, headache, vomiting and abdominal pain	MO	P	M	Basic hygiene training	R	M
11	MAP	May be associated with Crohn's disease: chronic inflammation of the bowel	MO	U	M	Basic hygiene training	R	M
12	Yersiniosis	Fever, abdominal pain and watery diarrhoea for 1-3 days	MI	U	L	Basic hygiene training	R	L
13	Corynebacteriosis	Can cause skin infections with lymphadenopathy	MI	R	L	Basic hygiene training	R	L
14	Clostridial Illness	*C. perfringens* enterotoxin can cause abdominal pain and diarrhoea Tetanus can result from wound infection	MO	R	M	Ante-mortem and post-mortem inspection removes animals with clinical signs from processing Basic hygiene training	R	M

No.	Specific Hazard	Description of outcome of hazard realisation	Inherent Risk*			Minimum Recommended Controls	Residual Risk#	
15	Bovine Tuberculosis	Ingestion can result in fever, abdominal discomfort and weight loss Inhalation causes more severe illness with chest pain and haemoptysis	MA	R	M	Eradicated from Australia Ante-mortem and post-mortem inspection removes animals with clinical signs from processing Basic hygiene training	VG	L

***Inherent risk categories: C** – consequences; **L** – likelihood; **IR** – inherent risk;
Residual risk categories: M – management control rating; **RR** – residual risk
Key to abbreviations in columns:
Consequences: CAT – catastrophic; MA – major; MO – moderate; MI – minor; I – insignificant
Likelihood: C – almost certain; L – likely; P – possible; U – unlikely; R – rare
Inherent risk: H – high; M – moderate; L – low
Management control: VG – very good; R – reasonable; P – poor
Residual risk: H – high; M – moderate; L – low

Table 10: Risks associated with Centrifugation stage								
No.	**Specific Hazard**	**Description of outcome of hazard realisation**	**Inherent Risk**			**Minimum Recommended Controls**	**Residual Risk**	
			C	**L**	**IR**		**M**	**RR**
1	Babesiosis	Fever, lethargy, anaemia, jaundice, haemoglobinuria, kidney failure	MA	R	M	Basic hygiene training Use of blood for human consumption	VG	L
2	Q Fever	'Flu-like symptoms for 7-10 days. Possible prolonged convalescence Possible chronic complications: hepatitis or endocarditis	MA	P	H	Basic hygiene training	P	H
3	Sarcosporidiosis	Usually asymptomatic; Possible nausea, abdominal pain and diarrhoea	MI	R	L	Basic hygiene training Use of blood for human consumption	VG	L
4	Campylobacteriosis	Fever, abdominal pains, diarrhoea. Rare chronic sequelae	MO	P	M	Basic hygiene training Use of blood for human consumption	VG	L
5	Salmonellosis	Mild fever, watery diarrhoea	MO	P	M	Basic hygiene training Use of blood for human consumption	VG	L
6	STEC	Mild to bloody diarrhoea for 7 days with severe abdominal pain Severe complications include HUS and TTP	MO	P	M	Basic hygiene training Use of blood for human consumption	VG	L
7	Leptospirosis	'Flu-like symptoms for 4-9 days with severe headache Meningitis may occur	MO	P	M	Basic hygiene training Use of blood for human consumption	VG	L

No.	Specific Hazard	Description of outcome of hazard realisation	Inherent Risk			Minimum Recommended Controls	Residual Risk	
8	Listeriosis	Mild 'flu-like conditions Can lead to septicaemia and encephalitis	MO	P	M	Basic hygiene training Use of blood for human consumption	VG	L
9	Pasteurellosis	Can cause respiratory illness	MI	U	L	Basic hygiene training Use of blood for human consumption	VG	L
10	Cryptosporidiosis	Mild diarrhoea with fever, headache, vomiting and abdominal pain	MO	P	M	Basic hygiene training Use of blood for human consumption	VG	L
11	MAP	May be associated with Crohn's disease: chronic inflammation of the bowel	MO	U	M	Basic hygiene training Use of blood for human consumption	VG	L
12	Yersiniosis	Fever, abdominal pain and watery diarrhoea for 1-3 days	MI	U	L	Basic hygiene training Use of blood for human consumption	VG	L
13	Corynebacteriosis	Can cause skin infections with lymphadenopathy	MI	R	L	Basic hygiene training Use of blood for human consumption	VG	L
14	Clostridial Illness	Perfringens enterotoxin can cause abdominal pain and diarrhoea Tetanus can result from wound infection	MO	R	M	Basic hygiene training Use of blood for human consumption	VG	M
15	Bovine Tuberculosis	Ingestion can result in fever, abdominal discomfort and weight loss Inhalation causes more severe illness with chest pain and haemoptysis	MA	R	M	Basic hygiene training Use of blood for human consumption	VG	L

Table 11: Risks associated with Handling Cell Fraction stage								
No.	**Specific Hazard**	**Description of outcome of hazard realisation**	**Inherent Risk**			**Minimum Recommended Controls**	**Residual Risk**	
			C	**L**	**IR**		**M**	**RR**
1	Zoonotic agents will be concentrated in the cell fraction	Illnesses as described above	MO	L	M	Use of blood harvested from cattle passed as fit for human consumption Hygiene training Use of PPE‡ – masks, gloves	R	M
2	Noxious gas (H_2S) produced as a result of faecal contamination of blood	Asphyxiation and death	CAT	U	H	Hygienic collection of blood limits contamination Good ventilation in blood handling areas	VG	M

‡ Preventative protective equipment

Table 12: Risks associated with Plasma Fractionation stage								
No.	**Specific Hazard**	**Description of outcome of hazard realisation**	**Inherent Risk**			**Minimum Recommended Controls**	**Residual Risk**	
			C	**L**	**IR**		**M**	**RR**
1	Babesiosis	Fever, lethargy, anaemia, jaundice, haemoglobinuria, kidney failure	MA	R	M	Basic hygiene training	VG	L
2	Q Fever	'Flu-like symptoms for 7-10 days. Possible prolonged convalescence Possible chronic complications: hepatitis or endocarditis	MA	U	M	Basic hygiene training	VG	L
3	Sarcosporidiosis	Usually asymptomatic; possible nausea, abdominal pain and diarrhoea	MI	R	L	Basic hygiene training	VG	L
4	Campylobacteriosis	Fever, abdominal pains, diarrhoea. Rare chronic sequelae	MO	U	M	Basic hygiene training	VG	L
5	Salmonellosis	Mild fever, watery diarrhoea	MO	U	M	Basic hygiene training	VG	L
6	STEC	Mild to bloody diarrhoea for 7 days with severe abdominal pain Severe complications include HUS and TTP	MO	U	M	Basic hygiene training	VG	L

No.	Specific Hazard	Description of outcome of hazard realisation	Inherent Risk			Minimum Recommended Controls	Residual Risk	
7	Leptospirosis	'Flu-like symptoms for 4-9 days with severe headache Meningitis may occur	MO	U	M	Basic hygiene training	VG	L
8	Listeriosis	Mild 'flu-like conditions Can lead to septicaemia and encephalitis	MO	U	M	Basic hygiene training	VG	L
9	Pasteurellosis	Can cause respiratory illness	MI	U	L	Basic hygiene training	VG	L
10	Cryptosporidiosis	Mild diarrhoea with fever, headache, vomiting and abdominal pain	MO	U	M	Basic hygiene training	VG	L
11	MAP	May be associated with Crohn's disease: chronic inflammation of the bowel	MO	U	M	Basic hygiene training	VG	L
12	Yersiniosis	Fever, abdominal pain and watery diarrhoea for 1-3 days	MI	U	L	Basic hygiene training	VG	L
13	Corynebacteriosis	Can cause skin infections with lymphadenopathy	MI	R	L	Basic hygiene training	VG	L
14	Clostridial Illness	C. perfringens enterotoxin can cause abdominal pain and diarrhoea Tetanus can result from wound infection	MO	R	M	Basic hygiene training	VG	L
15	Bovine Tuberculosis	Ingestion can result in fever, abdominal discomfort and weight loss Inhalation causes more severe illness with chest pain and haemoptysis	MA	R	M	Basic hygiene training	VG	L

Table 13: Risks associated with cleaning columns and ultrafiltration membranes								
No.	**Specific Hazard**	**Description of outcome of hazard realisation**	**Inherent Risk**			**Minimum Recommended Controls**	**Residual Risk**	
			C	**L**	**IR**		**M**	**RR**
1	Zoonotic agents will be concentrated in the residue	Illnesses as described above	MA	U	M	Hygiene training Use of PPE – masks, gloves	VG	L

Table 14: Risks associated with Spray Drying

No.	Specific Hazard	Description of outcome of hazard realisation	Inherent Risk			Minimum Recommended Controls	Residual Risk	
			C	L	IR		M	RR
1	Babesiosis	Fever, lethargy, anaemia, jaundice, haemoglobinuria, kidney failure	MA	R	M	Basic hygiene training	VG	L
2	Q Fever	'Flu-like symptoms for 7-10 days. Possible prolonged convalescence Possible chronic complications: hepatitis or endocarditis	MA	U	M	Basic hygiene training Use of PPE - masks	VG	L
3	Sarcosporidiosis	Usually asymptomatic; possible nausea, abdominal pain and diarrhoea	MI	R	L	Basic hygiene training	VG	L
4	Campylobacteriosis	Fever, abdominal pains, diarrhoea. Rare chronic sequelae	MO	U	M	Basic hygiene training	VG	L
5	Salmonellosis	Mild fever, watery diarrhoea	MO	U	M	Basic hygiene training	VG	L
6	STEC	Mild to bloody diarrhoea for 7 days with severe abdominal pain Severe complications include HUS and TTP	MO	U	M	Basic hygiene training	VG	L
7	Leptospirosis	'Flu-like symptoms for 4-9 days with severe headache Meningitis may occur	MO	U	M	Basic hygiene training	VG	L
8	Listeriosis	Mild 'flu-like conditions Can lead to septicaemia and encephalitis	MO	U	M	Basic hygiene training	VG	L
9	Pasteurellosis	Can cause respiratory illness	MI	U	L	Basic hygiene training	VG	L
10	Cryptosporidiosis	Mild diarrhoea with fever, headache, vomiting and abdominal pain	MO	U	M	Basic hygiene training	VG	L
11	MAP	May be associated with Crohn's disease: chronic inflammation of the bowel	MO	U	M	Basic hygiene training	VG	L
12	Yersiniosis	Fever, abdominal pain and watery diarrhoea for 1-3 days	MI	U	L	Basic hygiene training	VG	L

No.	Specific Hazard	Description of outcome of hazard realisation	Inherent Risk			Minimum Recommended Controls	Residual Risk	
13	Corynebacteriosis	Can cause skin infections with lymphadenopathy	MI	R	L	Basic hygiene training	VG	L
14	Clostridial Illness	*C. perfringens* enterotoxin can cause abdominal pain and diarrhoea Tetanus can result from wound infection	MO	R	M	Basic hygiene training	VG	L
15	Bovine Tuberculosis	Ingestion can result in fever, abdominal discomfort and weight loss Inhalation causes more severe illness with chest pain and haemoptysis	MA	R	M	Basic hygiene training	VG	L

6 Discussion

The greatest likelihood of exposure to zoonotic agents occurs in the early stages of the process, blood collection and centrifugation. During these stages of processing, the level of risk is similar to that present in normal cattle slaughter and dressing operations. At centrifugation, zoonotic agents would be concentrated into the cell fraction, and handling of this material would present a greater likelihood of exposure, and thus additional precautions such as Personal Protective Equipment (PPE) should be considered.

A number of zoonotic agents, carried in bovine faeces, are commonly present in the slaughterhall environment, and present a moderate risk to workers. Hygiene training will reduce the likelihood of illness occurring, as the route of infection is ingestion, but training *per se* was considered to be no more than reasonable control, as there is a strong reliance on the workers themselves complying with the personal hygiene measures.

Q fever, caused by *Coxiella burnettii*, remains a high risk in the early stages of processing, as the organism is excreted in the urine, uterine secretions, respiratory secretions and milk of cattle, aerosolised and acquired through inhalation. In a situation where live cattle are handled, the organism may be present and the workers exposed. In Australia, slaughterhouse workers have previously been routinely vaccinated against Q fever, and this additional control is to be recommended if the vaccine is available.

There have been fatalities arising from inhalation of noxious hydrogen sulphide (H_2S) gas produced by bacteria growing in blood storage tanks. Bacterial contamination arises through unhygienic blood collection, and bacterial growth occurs in such contaminated blood if it is not stored at low temperatures. H_2S can be produced by a wide variety of organisms, including members of the *Enterobacteriaceae*, which are present in faecal matter. The majority of these organisms are inhibited by low temperatures. For the production of bioactive agents from bovine plasma, it is recommended that the blood is collected hygienically, through a hollow knife closed system, and stored under refrigeration, as used for the collection of blood for human consumption. This will reduce the likelihood of bacterial contamination and subsequent outgrowth, and thus reduce the likelihood of H_2S production. Nevertheless, the blood and its fractions should be stored and handled in a well-ventilated area, and should be processed without undue delay.

Centrifugation should remove all cellular material from the plasma, including bacteria and intracellular organisms such as *Coxiella burnettii*. There is a small possibility that ruptured cells may release these organisms into the plasma, and that they may then be present at CSEP fractionation. Exposure to Q fever at this stage is considered to be unlikely, due to the inherent controls in the early stages of the process, so during fractionation it becomes a Low risk. Spray drying may aerosolise the organism, if present, so the use of PPE such as face masks could be considered to limit the potential for inhalation, or alternatively, staff could be vaccinated.

7 Conclusions

When blood is collected hygienically from cattle that are fit for human consumption, very few zoonotic agents remain present in the whole blood. Separation of the plasma from the cells is likely to confine any bacterial contaminants and intracellular organisms to the cell fraction. The plasma produced is unlikely to carry zoonotic pathogens. *Babesia* and *Coxiella* organisms may be released into the plasma if the host cells are ruptured, but careful handling of the blood should prevent this occurring.

This Risk Assessment provides a guide to the zoonotic hazards that may be encountered when processing bovine blood for the production of bioactive agents. The report finds that:

1. The risk to workers presented by zoonotic agents is greatest in the early stages of the process (Table 15). These risks are at a level similar to those encountered in routine beef production.
2. Centrifugation reduces the risks associated with plasma fraction, however this could concentrate any zoonotic agents in the cell fraction if they are present.
3. The likelihood of zoonotic agents being transmitted through the process and into the product is very low, as most, if not all, should be removed at centrifugation.
4. This Assessment makes certain assumptions with regard to the susceptibility of workers and the presence of zoonotic agents in cattle which may or may not apply to individual processors or meatworks.

8 Recommendation

Only blood that is fit for human consumption should be used for separation and fractionation. The animals from which the blood is derived should pass both ante-mortem and post-mortem inspection procedures, and the blood should be collected hygienically and subsequently handled and stored in such a manner as to prevent contamination.

Blood that is to be used for human consumption should be collected hygienically, in a manner that minimises the risk of contamination from extraneous sources. Blood could be collected simply by placing a container directly below the sticking wound, but microbial contamination could occur from regurgitated rumen content, from the hide, or from airborne contamination. Hygienic collection of blood through a closed system is preferred, in which an area of hide is carefully removed to expose the sticking site, and a hollow-bladed sticking knife (“vampire-knife”) inserted into the major blood vessels leaving the heart. The blood is transferred via an enclosed tube to the holding tank. Blood should not be collected by suction, as this will rupture cells.

Blood for edible purposes must remain traceable to the individual carcass of origin, or to a specific batch of carcasses, until all carcasses are inspected and deemed fit for human consumption. If any carcass is rejected as unfit for human consumption, the blood relating to that carcass, and any other blood with which it is mixed must also be rejected, and the entire blood collection system from knife to holding tank must be properly sanitised prior to re-use.

Blood should be centrifuged immediately after collection, before chilling, as chilling can cause haemolysis, or rupture of the red blood cells, which releases cell contents into the plasma. The plasma fraction should then be chilled to below 4°C for storage.

Zoonotic pathogens may be concentrated in the cellular fraction, so care should be taken when handling this fraction prior to disposal or processing. Workers should be made aware

of the hazards, and personal protective equipment such as face masks and gloves should be worn. The area in which blood is separated should be well ventilated.

Specific recommendations:

1. Processors adopting the CSEP process to fractionate bovine blood must conduct their own Risk Assessment of their own specific conditions pertaining to their operation.
2. Where a processor-specific Risk Assessment rates the residual risk as Moderate or High, additional control measures such as protective clothing are recommended.
3. Safety precautions are recommended for workers handling the cell fraction residue after centrifugation.

9 Further work

This Risk Assessment is a qualitative assessment, and rates the hazards relative to one another. In order to carry out a quantitative assessment of the risks involved in bovine blood fractionation, further research would be required into the prevalence of the zoonotic agents and the dose-response characteristics of the agents.

Table 15: Effect of process hurdles on the likelihood of zoonotic agents being present in bovine plasma

Illness	Hurdle National surveillance and eradication program	Ante-mortem and post-mortem inspections	Hygienic collection of blood	Centrifugation
Bovine Babesiosis		Clinical cases blocked*		Good reduction
Q fever				Good reduction
Sarcosporidiosis				**Blocked**
Campylobacteriosis			Good reduction	**Blocked**
Salmonellosis		Clinical cases blocked	Good reduction	**Blocked**
STEC			Good reduction	**Blocked**
Leptospirosis		Clinical cases blocked	Good reduction	**Blocked**
Listeriosis		Clinical cases blocked	Good reduction	**Blocked**
Pasteurellosis		Clinical cases blocked	Good reduction	**Blocked**
Cryptosporidiosis			Good reduction	**Blocked**
MAP			Good reduction	**Blocked**
Yersiniosis			Good reduction	**Blocked**
Corynebacteriosis		Clinical cases blocked	Good reduction	**Blocked**
Anthrax		**Blocked**		
Clostridial illness		Clinical cases blocked	Good reduction	**Blocked**
Bovine Brucellosis	**Blocked***	**Second Block**		
Bovine Tuberculosis	**Blocked**	**Second Block**		
Trypanosomiasis	**Blocked**			
Viral Encephalitis diseases	**Blocked**			
Rabies	**Blocked**			

*__Blocked__: Animals or products containing the zoonotic agent should not be able to progress further along the processing chain.

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