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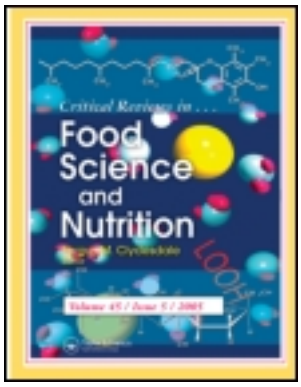

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Issues Related to the Use of Blood in Food and Animal Feed

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Blood has traditionally been used as a high protein ingredient in both human food and animal feed, with resulting economic, environmental and nutritional benefits. However, potentially serious health and safety issues related to blood consumption, particularly the risk of pathogenic or harmful metabolic materials, the infectivity of prion diseases, and the presence of identified allergens such as bovine serum albumin (BSA), are causing many consumers to shy away from any product containing either animal blood or ingredients derived from animal blood. Thus, despite the significant volumes of blood produced by slaughterhouses, blood is currently underutilized as a food ingredient. This article reviews the use of animal blood as an ingredient in food intended for human consumption or for animal feed and discusses the related consumer concerns.

Keywords Plasma, red blood cells, proteins, BSE

1. INTRODUCTION

Animal blood is the first byproduct obtained after slaughter and is widely used in food preparations for human consumption, as a feed ingredient for animals, and in a variety of laboratory, medical, industrial, and agricultural applications. The food industry currently uses about 30% of slaughterhouse blood (Gatnau et al., 2001), with most going to the meat industry for use as a gelling agent and natural colorant. The remainder of the blood produced is utilized by the pet food, livestock, agriculture, pharmaceutical, medical, diagnostic, and paper industries. Blood constitutes 3–5% of the live weight of animals (Halliday, 1973) and approximately 50% of this can be collected at slaughter, with the remainder being retained in the capillary system (Wanasundara et al., 2003).

Blood and blood products that are used as food sources for both humans and animals are generally obtained from bovine and porcine sources, as the use of blood from other species is seldom practicable (Karasz et al., 1976). The disposal of unused blood is often a serious problem: in the United States (US) alone, the annual blood waste is thought to be in the region of 1.6 million tons and the large quantities of blood produced, coupled with its high solids content (18%), and significant chemical oxygen demand (COD) (500,000mg O_2/L) (Del Hoyo et al., 2007), has serious environmental implications. The disposal of untreated blood by spraying it on to agricultural lands or dumping it into municipal sewage systems, as was done in the past, has now been banned (Nowak and von Mueffling, 2006). Disposing of blood (and other slaughterhouse byproducts) in an environment friendly manner is expensive and the cost continues to rise (Liu, 2002), adding to the cost of meat production. The selling price of the carcass alone cannot adequately compensate for the high capital input associated with raising the animal, so finding ways to utilize byproducts such as blood not only increases profits and sustains the meat industry but also reduces the problems associated with its disposal.

Bovine blood typically consists of 80.9% water, 17.3% proteins, 0.23% lipids, 0.07% carbohydrates, and 0.62% minerals (Duarte et al., 1999). Thus, in addition to the environmental and economic benefits that derive from utilizing blood, its recovery provides a low cost but high quality source of protein and other nutrients for both humans and animals. This is particularly relevant in developing countries, where protein deficiency malnutrition is a major problem because many people cannot afford to buy high quality protein products in the form of meat and poultry. Continuing efforts to utilize blood and blood proteins in the food and feed chain can therefore result in major environmental, economic, and nutritional benefits. However, consumer concerns, including the fear of contracting bovine spongiform encephalopathy (BSE), potential allergic reactions to blood proteins, and the belief that blood drained from animals contains harmful microorganisms, toxins, and

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metabolites, militates against efforts to fully utilize blood proteins as a food source. This article reviews how these proteins are used in the food and feed chain, and examines the potential health risks posed by their usage in light of these consumer concerns.

2. ANIMAL BLOOD AS HUMAN FOOD

Whole animal blood has traditionally been used in Europe and Asia in such products as blood sausage, blood pudding, blood cake, and blood curd (Liu, 2002). Although these products are not commonly found in US supermarkets, blood and blood proteins do find their way into the US food chain, usually in the form of blood protein ingredients processed from the plasma or cellular fraction of blood. Examples of blood protein ingredients that are available commercially are shown in Table 1. The US Meat Inspection Act approves the use of blood in food provided that it is obtained by bleeding an animal that has been inspected and passed for use as meat (9CFR 310.20). These blood proteins have excellent functional properties and nutritive value which are made use of in human food preparation (Mandal et al., 1999; Ockerman and Hansen, 2000; Silva and Silvestre, 2003).

2.1 Whole Blood

Blood is made up of two fractions, namely the cellular fraction (red blood cells, white blood cells, and platelets) and the plasma fraction, with the former suspended in the latter. About 18% of animal blood is composed of protein, with hemoglobin in the red blood cells accounting for more than 50% of the total protein content (Liu et al., 1996). Whole blood is used in the manufacture of blood sausages which are popular in many European countries. In ordinary sausages blood or blood proteins are sometimes added as a color enhancer, filler or binder, and may also be used as a casing (Caldironi and Ockerman, 1982; Stiebing, 1990; Ockerman and Hansen, 2000; Santos et al., 2003). Whole blood is also used as a meat extender in meat products, thus lowering the cost without compromising the nutritional value (Heinz and Hautzinger, 2007).

The amount of whole blood used in meat and other food products tends to be relatively low as increasing the proportion has a detrimental effect on sensory qualities, particularly flavor and color (Ockerman and Hansen, 2000). In sausage products, for example, the blood content is restricted to 0.5 to 2% of the sausage component. Restricting the addition of blood to within this range is reported to improve the overall perception of color and meat taste compared to reference samples with no blood added (Slinde and Martens, 1982). Heme iron is better absorbed than nonheme iron, so the high heme iron content of bovine blood has led to it being used to fortify commonly consumed iron-deficient foods as a measure to combat iron deficiency anemia (IDA) in humans, a global malnutrition problem (Walter et al., 1993; Kikafunda and Sserumaga, 2005). In addition to its use as a food ingredient, animal blood is also utilized as a processing aid in some types of food production. For example, bull's blood is used as a coagulant to clarify wine before bottling (Ockerman and Hansen, 2000).

2.2 Plasma and Derived Products

Plasma, which makes up 65–70% of the total volume of whole blood (Halliday, 1973), contains about 7.9% protein which is made up principally of immunoglobulins (4.2%), albumins (3.3%), and fibrinogen (0.4%) (Howell and Lawrie, 1983). The plasma proteins fibrinogen and thrombin are selectively cryo-precipitated from plasma and used as a natural binder in whole meat processing. This product is patented by Harimex B.V., Netherlands, and is sold commercially under the brand name Fibrimex® (Wanasundara et al., 2003). Plasma proteins are also used as emulsifiers in emulsified meat systems (Mittal, 2005), as protein supplements and fat replacers in products such as sausages, and as an inhibitor for endogenous proteases in surimi-type products made from certain species of fish (Wanasundara et al., 2003; Viana et al., 2005). Although plasma proteins are used mainly in meat processing, they are also found

Table 1 Examples of food grade proteins produced from blood and blood fractions

Product	Description	Usage	Company
ImmunoLin	Serum concentrate	Dietary supplement to be added to bars and drinks to boost immune system	Proliant Inc., USA
NutraGammax	Serum protein isolate	Dietary supplement targeting the sports nutrition world	Proliant Inc., USA
Fibrimex	Isolate of thrombin and fibrinogen precipitated from plasma	Natural binder for whole muscle processing	Harimex B.V., Netherlands
Harimix P	Hemoglobin	Natural meat colorant	Sonac B.V., Netherlands
Plasma powder FG	Plasma	Binder in meat products	Sonac B.V., Netherlands
Prietin	Whole blood	Making Morcilla (blood sausage)	Lican Functional Protein Source, Chile
Vepro globin	Globin	Emulsifier in meat products	VEOS Group, Belgium
Proferrin ES	HIP	Dietary supplement for the treatment of iron deficiency	Colorado Biolabs Inc., USA
Proferrin Forte	HIP plus folic acid	Dietary supplement for the treatment of iron deficiency	Colorado Biolabs Inc., USA

in some nonmeat products. For example, they are often used as a lowcost substitute for egg products in the bakery industry (Raeker and Johnson, 1995; Mandal et al., 2000; Ockerman and Hansen, 2000; Fernandez-Michel et al., 2006). Spray-dried blood plasma has been incorporated into biscuit flour to provide a cheap high protein flour alternative to durum wheat flour for the production of protein-rich pasta (Yousif et al., 2003). A plasma product manufactured by Proliant Inc., Ankeny, IA, and sold under the brand name Immunolin® is widely available to the sports nutrition world as an immune function booster (Table 1, www.proliantinc.com/health/products/sports-nutrition.asp).

2.3 Red Blood Cells (RBCs) and Derived Products

The use of RBCs and derived products as food ingredients is limited because the heme component of hemoglobin, the major protein in RBCs (and in blood), imparts an undesirable color, odor, and metallic taste to the final product (Duarte et al., 1999; Liu et al., 1996). The presence of even trace amounts of hemoglobin imparts a dark-brown color to foods (Yang and Lin, 1998). Hemoglobin-containing products are therefore usually restricted to traditional dark colored products such as black sausage, black pudding, and blood cookies (Ockerman and Hansen, 2000). To make RBCs more acceptable and useful as food ingredients, the heme pigment can be removed through various techniques (Tybor et al., 1975; Sato et al., 1981; Autio et al., 1984; Hayakawa et al., 1986; Houlier, 1986; Yang and Lin, 1996; Duarte et al., 1999) to produce an off-white product that is known in the food industry as globin or decolorized blood. This is sometimes used as a fat replacer in meat products (Viana et al., 2005). Because of its richness in essential amino acids, particularly lysine, globin has also been used to fortify cereal-based products as these are usually deficient in lysine (Landmann et al., 1980). Heme iron polypeptide (HIP), a soluble heme moiety with an attached polypeptide obtained by the enzymatic digestion of bovine hemoglobin, is currently available in the United States (US) under the brand name Proferrin® and used for the treatment of IDA (Table 1). Another use of the RBC fraction of blood is to produce a synthesized product from bovine RBCs known as cooked cured meat pigment (CCMP) (dinitrosyl ferrohemochrome). CCMP has been suggested as a potentially suitable alternative for the meat curing agent nitrite, which is associated with carcinogenic N-nitroso compounds (Shahidi et al., 1985; Shahidi and Pegg, 1991).

3. CONCERNS ASSOCIATED WITH THE USE OF BLOOD IN FOOD

The utilization of these blood proteins as food ingredients thus offers considerable nutritional, economical and environmental benefits. It is vital, however, to regulate their use in terms of both labeling and enforcement in order to prevent their fraudulent use and also address the concerns of those who prefer to avoid such products. At present, processed food product labels do not always clearly specify the source of all food ingredients. Certain religions, for example Islam and Judaism, require their adherents to abstain from consuming any material derived from blood, while vegetarians and vegans avoid such products for ethical reasons. A further section of the population prefer not to eat blood proteins for health reasons, either due to the notable allergens in blood or their belief that blood contains toxic metabolites that render it unsafe for human or animal consumption. Unambiguous labeling and enforced regulatory action will boost consumer confidence in using these products. This section addresses these issues.

3.1 Allergy to Blood Proteins

Bovine serum albumin (BSA), a 69kDa globular protein, is the most abundant (50–60%) protein in the plasma fraction of bovine blood (Davila et al., 2007). BSA is also found in beef and cow's milk, crossing the capillary endothelia from plasma into the muscle and mammary gland by transcytosis (Tuma and Hubbard, 2003; Monks and Neville, 2004), and is therefore a convenient marker for estimating the transfer of blood proteins to milk (Guidry et al., 1980a; Guidry et al., 1980b). The concentration of BSA in milk is only about 1/200 that in blood (Poutrel et al., 1983) but it has been implicated in allergic reactions to milk (Goldman et al., 1963a; Goldman et al., 1963b; Martelli et al., 2002; Wal, 2002) and also beef (Fiocchi et al., 1995; Kanny et al., 1998; Fiocchi et al., 2000; Han et al., 2000; Takahata et al., 2000). Beef allergy, the most common of the meat allergies, is found in between 1.5 and 6.5% of children suffering from atopic dermatitis or food allergies (Fiocchi et al., 1995; Werfel et al., 1997; Rance et al., 1999), and in 20% of the children allergic to cow's milk (Werfel et al., 1997). A recent study by Martelli et al. (2002) indicates that many individuals with cow milk allergy (CMA) may also be allergic to beef. In this study, out of 28 children with diagnosed cases of beef allergy, 26 (92.9%) were also found to be allergic to cow's milk using a skin prick test and a double-blind placebo-controlled food challenge. Serum albumins from other species have also been associated with allergies to meat, including lamb (Palacios Benito et al., 2002), pork (Llatser et al., 1998), and rabbit (Palacios Benito et al., 2002). Similarly, allergies to the serum albumin in milk from species such as sheep have been reported (Asero et al., 2009). Though meat allergy seems to be a problem mainly among children, instances of meat allergy in adults have been discussed in the literature. For example, Kanny et al. (1998) reported a case of beef allergy in a 19-year-old woman, where BSA was found to be the culprit allergen. Immunoglobulin, a protein present in both blood and milk, has also been implicated in cases of meat allergy (Werfel et al., 1997; Ayuso et al., 2000; Han et al., 2000), while bovine IgG has been suggested as an important allergen in CMA (Maeda et al., 1993).

Despite the involvement of the same blood proteins in milk and meat allergies, there have been no reports in the literature of

allergies caused by exposure to blood proteins via their use as food ingredients. This may be in part because of the heat-labile nature of these blood protein allergens (Werfel et al., 1997; Fiocchi et al., 1998; Vicente-Serrano et al., 2007); their concentrations are greatly reduced by the spray-drying process that is part of their commercial production. This reduced allergenicity due to heat processing may explain why in most cases of beef allergy, allergic responses are to raw or medium rare cooked beef but not meat that has been fully cooked. Industrial heat processing is more efficient than domestic cooking in this respect (Fiocchi et al., 1998; Fiocchi et al., 2000) and the inclusion of small amounts of spray-dried blood proteins in meat products further reduces their allergenicity after cooking. The use of different matrices (milk, beef, and blood) to carry these identified allergenic proteins (immunoglobulins and BSA) may also explain why these allergens have been implicated in meat and milk allergies but not in blood protein allergy. The sensitivity of BSA to heat has been reported to vary considerably in different matrices. For example, BSA is more heat-sensitive in colostrum than in mature milk (Hanson and Mansson, 1961). These differences in heat-sensitivity may be why BSA is considered a minor allergen in milk but a major allergen in beef. Although the heat-sensitivity of BSA in blood has not been well studied, it could be more heat-labile than the allergenic proteins in milk and meat, hence the lack of reports of allergic reactions associated with the use of blood proteins as food ingredients in the literature. Some of these proteins are subjected to freeze-drying rather than spray-drying, and freeze-dried blood products are likely to present an even lower risk of inducing allergic reactions; one study found that although some participants reacted to heat-treated beef, none reacted to freeze-dried beef (Fiocchi et al., 1998). The faint possibility that they might trigger allergic reactions is minimal, with no reported instances in the literature. Thus, the presence of allergenic proteins such as albumins and globulins in blood seems to constitute virtually a zero threat of allergic responses in sensitive individuals through their use as food ingredients.

3.2 Belief that Blood is Harmful

Some people avoid consuming food products containing blood as they believe that it contains harmful microorganisms and toxins and toxic metabolites that render them unsafe for human consumption. The concern that the blood may be a source of dioxin is almost certainly unfounded as analyses of animal blood protein samples have never shown detectable amounts of dioxin (Gatnau et al., 2001). This is not surprising; dioxin accumulates in fat and the fat content of blood proteins is very low. However, foods of animal origin are easily contaminated with spoilage microorganisms and possibly pathogens through improper processing and handling (Al-Bachir and Mehio, 2001). Blood taken from a healthy animal is essentially sterile, so any contamination is due to the bleeding technique and the drainage system employed during collection (Riaz, 2010). The concern that blood is a haven for pathogens and toxins can therefore be nullified if the necessary measures are in place to prevent contamination. The collecting systems for blood currently used in the industry fall into two major categories: open- and closed-draining systems. Open-draining systems cannot guarantee the absence of hazards in the collected blood and blood collected through this method tends to have a high microbial load as it comes into contact with surface organisms on the carcass, wash water, vomit, feces, and so on. A closed-draining system, on the other hand, allows the hygienic collection of blood and ensures that the blood collected has a low microbial count.

Blood to be used for human consumption must therefore be collected by a closed-draining system that utilizes a hollow knife to collect the blood directly from the throat of the slaughtered animal and deposits it directly into a refrigerated vessel. The area where the incision will be made is first excised with a sterile knife to expose a clean area of tissue (CSIRO, 2003). Blood usually flows into the holding tank by means of gravity; vacuum may also be employed to aid in bleeding, although this sometimes causes the blood vessel to collapse, blocking the flow of blood into the hollow knife (CSIRO, 2003). This system of blood collection ensures that blood is siphoned from the animal into collecting tanks without exposure to the atmosphere. Both regulators and manufacturers have instituted stringent measures to ensure that animal blood intended for use as a food ingredient is as safe as possible. Only authorized slaughterhouses are allowed to collect blood that will enter the human food chain and only blood that has been hygienically collected is allowed to be processed into food-grade blood proteins after inspection and approval by government veterinarians. In the US, federal regulation 9 CFR 310.20 monitors blood that is to be used in human food, ensuring that it originates from official establishments whose livestock and carcasses have been inspected and passed. After collection, the blood is immediately refrigerated at 4°C and stored in stainless steel storage tanks at the abattoir. The blood is continuously stirred in these tanks and is transported to the manufacturing plant in dedicated isothermal stainless steel trucks. On arrival at the manufacturing plant, quality assurance (QA) and quality control (QC) procedures are performed on the received blood. Blood that passes the QA and QC inspections is then unloaded and carried through closed systems into refrigerated and stirred stainless steel tanks for subsequent processing into food-grade blood proteins (Gatnau et al., 2001).

To prevent uninspected blood from entering the human food chain, the collected edible blood is associated with individual animals or batches of animals from which the blood was sourced. At the abattoir, blood collected from individual animals may be held in containers mounted on carousels that travel alongside their respective carcasses down the slaughter floor. Alternatively, blood from several animals may be accumulated and held until all the carcasses from which it was drawn have been inspected. If any carcass fails to pass the inspection, all the blood collected within that period is discarded (CSIRO, 2003). This procedure ensures that only blood sourced from healthy animals and collected hygienically under the close supervision

of the regulatory agencies enters the human food chain. Hence, utilization of blood proteins in the human food chain poses a minimal safety threat. Spray-drying at high temperatures, which is part of the subsequent processing, inactivates any remaining viruses and bacteria, further improving the safety of these products.

It is important to remain vigilant, however, as even if blood is collected hygienically postharvest microbial contamination could still lead to problems, especially when used in a nonpowder form such as liquid plasma, due to its high nutrient content and high water activity. Slaughter plants in some parts of the world have therefore instituted QA programs to control chemical, physical, and biological hazards during slaughter. In one study designed to investigate this issue, RBCs, serum, albumin, and immunoglobulin fractions separated from blood collected from a HACCP-implemented plant were reported to have excellent microbiological quality (Ramos-Clamont et al., 2003). Therefore, making it mandatory for blood protein manufacturers to develop HACCP systems should help boost consumer confidence in the safety of these products.

4. ANIMAL BLOOD AS FEED

Blood and plasma proteins have also been utilized as high quality ingredients in food for both pets and farm animals due to their nutritional and health benefits (Drepper and Drepper, 1979; Cozzi et al., 1995; Ockerman and Hansen, 2000; Lawrence et al., 2004; Polo et al., 2004), replacing increasingly expensive traditional protein sources (Cozzi et al., 1995). Spray-dried animal plasma is added to swine starter diets to increase feed intake and growth (Coffey and Cromwell, 2001). Feeding weanling piglets blood immunoglobulins not only accelerates daily weight gain, but also reduces the incidence of scours and mortality (Lee et al., 1987). Spray-dried animal plasma is also widely used in loaf, chunk, and pouch type pet foods to enhance the texture of the product by improving cohesion between the different ingredients in the recipe, taking advantage of its excellent water binding and emulsifying capacity. The use of spray-dried animal plasma in pet food not only enhances its palatability but is also preferred by carnivores such as cats (Polo et al., 2005; Polo et al., 2007).

More recently, blood proteins have been used as a whey protein substitute in milk replacer for dairy calves. In the US, dairy calves are fed milk replacer during their pre-weaned period in place of whole milk for a variety of reasons. Economically, it is cheaper to use milk replacer, which is a byproduct of the milk manufacturing industry, than saleable whole milk. The practice is also more convenient as milk replacers allow farmers to select from an array of ingredients to manipulate protein, fat, and vitamin levels in the milk replacers in order to enhance calf growth. Calves tend to be particularly vulnerable to diseases that are transmitted from cow to calf through the feeding of unpasteurized milk, so disease prevention is another factor contributing to the rising popularity of milk replacers. Whey has traditionally been the major protein ingredient in milk replacers, but as the cost of whey products continues to rise as a result of increasing demand for its use in human foods, there is now an urgent need for alternative protein sources for milk replacers. Blood derived proteins such as spray-dried bovine and porcine plasma (Quigley and Wolfe, 2003) and spray-dried hydrolyzed red blood cells (Quigley et al., 2000) are therefore finding new uses as cheap protein sources to substitute for whey products in milk replacers. Spray-dried hydrolyzed red blood cells can replace up to 43% of whey proteins without adverse effects on the performance of animals (Quigley et al., 2000). The inclusion of spray-dried plasma in milk replacers not only reduces costs but also reduces morbidity and mortality in calves (Quigley and Wolfe, 2003).

As aquaculture becomes an increasingly important source of food for human consumption, recent research has looked at using blood meal in lieu of more expensive fish meal. Fish require a high proportion of protein in their diet because they metabolize protein as a source of energy and fish meal has traditionally been the main protein source in aquaculture feeds as it is an excellent protein source (Agbebi et al., 2009). Generally, the fish species menhaden and anchovies are used in the manufacture of fish meal, although herring is also used to a lesser extent (Meeker, 2009). However, the limited supply of these raw materials, coupled with the increasing cost of fish meal, makes it imperative to identify alternate protein sources to replace or supplement fish meal in aquaculture diets. Plant proteins are not a useful alternative as they contain anti-nutritional factors that negatively affect fish growth, nutrient utilization and general well being (Francis et al., 2001). The marked differences in amino acid composition between plant proteins and fish proteins (and hence fish meal) are thought to lead to deficiencies in essential amino acids that reduce growth and protein utilization in fish unless the diet is supplemented (Espe et al., 2006; Olsen et al., 2007). Blood meal provides a cheap and effective replacement for fish meal in fish diets, wholly replacing fish meal with no adverse effect on growth, survival, and feed conversion ratio (Agbebi et al., 2009; Otubusin et al., 2009)

5. CONCERNS ASSOCIATED WITH THE USE OF BLOOD IN FEED

The main concern regarding the use of blood in animal feed is the risk of transmitting BSE, colloquially known as “mad cow disease”, which was first diagnosed in cattle in the United Kingdom (UK) in 1986 (Wells et al., 1987). BSE belongs to a group of related fatal progressive degenerative diseases known as transmissible spongiform encephalopathies (TSEs) that affect the central nervous system (CNS) in both humans and ruminant animals. Examples of TSEs include Creutzfeldt-Jakob disease (CJD) in humans (Creutzfeldt, 1920; Jakob, 1921), transmissible mink encephalopathy (TME) in mink (Hartsough and Burger, 1965), chronic wasting disease (CWD) in deer and elk (Williams and Young, 1980), scrapie in sheep and goats (where it was described as far back as the 17th century). It is generally

accepted that the infectious agent responsible for TSEs is an abnormal form of a naturally occurring protein called a prion. Prion proteins are normally found on the surface of nerve cells and lymphocytes. When an abnormal prion (PrP^{sc}) comes in contact with a normal prion (PrP^{c}), the normal prion converts to the abnormal form. In the 1990s, a new variant form of CJD, termed vCJD, was discovered (Will et al., 1996a). vCJD shares some common features with CJD namely dementia, ataxia, and myoclonus (Will et al., 1996a; Will et al., 1996b), but differs from CJD in other respects. Evidence now indicates that the prion responsible for BSE shares common features with the prion responsible for vCJD. The outbreak of BSE in the UK has been attributed to changes in cattle farming over the last several decades, during which time animals have increasingly been fed mammalian proteins to increase output (Chalus and Peutz, 2000); while vCJD was attributed to the consumption of BSE-infected beef.

5.1 Efforts to Control the Spread of BSE

So far, no natural cases of TSEs have been found in non-ruminants such as horses and pigs (Matthews and Cooke, 2003; Lipp et al., 2004), In 1997, the US FDA (Food & Drug Administration) banned the use of ruminant proteins in ruminant feed, the so-called "ruminant feed ban", in order to prevent the establishment and spread of BSE in ruminants in the US (21 CFR 589.2000). Blood, pure porcine and equine products, and milk products were, however, exempted because the FDA believed that they represented only a minimal risk of transmitting TSEs to ruminants through feed. A similar ban had already been introduced in the UK in 1988 (SI 1988/1039) and throughout the European Union (EU) in 1994 (94/381/EC). In 2000, the use of all animal proteins, including blood, in animal feed was banned in the EU (2001/9/EC) to further prevent the spread of BSE and ensure food safety. Currently in the EU, porcine blood meal can be used in fish feed (EC1234/2003) and porcine blood products are allowed to be fed to nonruminant farm animals (EC1292/2005). Because of their awareness of the risk of BSE, consumers are increasingly demanding beef from cattle that has been raised under natural living conditions. This has led to a search for new kinds of plant materials to replace animal proteins as feed ingredients for ruminant livestock (Brambilla and De Filippis, 2005). However, because plant materials have lower protein levels than blood proteins, lack essential amino acids, and contain antinutritional factors, many farmers still prefer to use blood proteins, although from a porcine rather than a ruminant source. There has been some debate whether ruminant blood should be banned or allowed in animal feed, as a number of studies have shown blood may carry the infectious agent, prions, in TSEs.

5.2 Blood Infectivity of TSEs

In naturally TSE infected animals, including sheep and goats with scrapie, mink with TME, and cows with BSE, all attempts to transmit disease (TSEs) through the inoculation of blood have failed (Brown, 2000). Epidemiological data have failed to reveal a single case of sporadic CJD that could be ascribed to the administration of blood or blood products among patients with CJD, or among patients with hemophilia and other congenital clotting or immune deficiencies who have received repeated doses of plasma concentrates (Brown, 2000; Houston et al., 2000; Flan and Arrabal, 2007). Several animal experiments (Table 2) have also demonstrated that blood does not carry infectivity in TSE transmission (Fraser et al., 1992; Middleton and Barlow, 1993; Wells et al., 1998; Bradley, 1999; Wells et al., 2003; Espinosa et al., 2007).

In stark contrast, transfusion related cases of vCJD transmission in humans (Llewelyn et al., 2004; Peden et al., 2004; Anonymous, 2006; Anonymous, 2007) clearly implicate blood as carrying infection in TSEs. To date, four cases of vCJD disease associated with blood transfusion have been reported (Table 3). All four cases had received transfusions of nonleucodepleted red blood cells between 1996 and 1999 (Anonymous, 2007). Several animal experiments (Brown et al., 1999;

Table 2 Animal experiments that do not support the infectivity of blood in TSE infection

Donor	Recipient	Source of infectious agent	Inoculation route	Tissues that caused infectivity or showed infectivity	Reference
BSE infected cow	Mice	Brain homogenate	Intracerebral and intraperitoneal	CNS	(Fraser et al., 1992)
BSE infected cow	Cattle	Brain stem	Oral	CNS Dorsal root ganglia Trigeminal ganglion	(Wells et al., 1998)
BSE infected cow	Cattle and mice	Brain, spleen, & lymph nodes	Parenteral	Brain	(Bradley, 1999)
BSE infected cow	Mice	Brain, spleen, placenta, mammary gland, suprammary and mesenteric lymph nodes	Oral	Brain	(Middleton and Barlow, 1993)
BSE infected cow	Piglets	Brain homogenates	Intracraneal, intravenous, and intraperitoneal	CNS and alimentary tissues	(Wells et al., 2003)
BSE infected cow	Cattle	Brain stem	Oral	CNS, tonsils, Peyer's patches	(Espinosa et al., 2007)

Table 3 Transfusion cases implicating blood in human TSE transmission

Case	Patient	Donor	Reference
1	Developed vCJD six and half years after receiving transfusion of red blood cells	Developed symptoms of vCJD 3 years and 4 months after donating the blood and died in 2000 of pathologically confirmed vCJD	Llewelyn et al., 2004
2	Received red blood cells from a donor and died 5 years later from causes unrelated to vCJD. Post mortem examinations found abnormal prion protein in the spleen and cervical lymph node but not in the brain, and no pathological features of vCJD were found	Donor developed symptoms of vCJD eighteen months after donation and died 2 years later of autopsy confirmed vCJD	Peden et al., 2004
3	Developed vCJD almost eight years after receiving a transfusion of red blood cells	Developed vCJD about 20 months after donating this blood	Anonymous, 2006
4	Developed symptoms of vCJD eight and a half years after receiving a transfusion of red blood cells	Developed vCJD about 17 months after donating this blood. The donor in this case was also the donor in the third case.	Anonymous, 2007

Houston et al., 2000; Taylor et al., 2000; Hunter et al., 2002; Siso et al., 2006) have also demonstrated the infectivity of blood in TSEs (Table 4). The role of blood in transmitting TSEs is further supported by the biochemical detection of PrP^{sc} in blood for the first time (Castilla et al., 2005) and by reports that vCJD, unlike CJD, has consistently been detected in blood-interactive lymphoreticular tissues (Hill et al., 1999).

This controversy surrounding blood infectivity in TSEs may be attributable to our incomplete understanding of the precise tissue distribution of PrP^{sc} in TSE infection, the route of infection, and the lack of sufficiently sensitive tests for TSEs. In general, the spectrum of tissues harboring the infection partly depends on the "strain" of prion involved and each strain exhibits a distinct preference for the host animal and type of cell in which it replicates. Host factors also seem to control the tissue tropism. For example, despite widespread habitation of extraneural tissues in vCJD, BSE prions are largely confined to the neural compartment of cows, even after oral exposure (Aguzzi and Glatzel, 2004). New findings, however, are continually changing our perceptions regarding the tissue distribution of PrP^{sc} in TSEs. For example, extraneural tropism was originally thought to set the vCJD strain apart from CJD. However, findings by Glatzel et al. (2003) indicate that prions can also accumulate in the lymphoid organs and skeletal muscle of CJD patients. Prior to this report, muscle had never been reproducibly shown to contain the infectious agent for any form of TSE, whatever the affected species (Brown et al. 2001).

Taken together, the evidence now suggests that the tissue distribution of the abnormal prion in TSE infection is wider than initially thought, which suggests that blood may indeed carry infectivity in BSE. The failure to detect infectivity in blood may therefore be due to the route of infection and the insensitivity of existing tests, while differences in tissue tropism may be explained by the route of infection. For example, intraperitoneal introduction of prions in rodents is followed by prion replication in the thoracic spinal cord (Kimberlin and Walker, 1982), while, in hamsters orally infected with scrapie, vagal colonization is preferred (Beekes and McBride, 2000). As BSE is transmitted through oral exposure to the infectious agent by way of the animal's feed, it is therefore likely that the levels of the agent in the blood will be relatively low, as oral challenge favors vagal colonization.

According to Dickmeiss and Gerstoft (2002), the in vitro assays used at the time were insufficiently sensitive and thus of little use in studies of blood infectivity in TSEs. It was, therefore, not surprising when researchers Castilla et al. (2005) reported the detection of PrP^{Sc} in blood, representing the first time PrP^{Sc} had been detected biochemically in blood. They reported that the concentration of the infectious agent in blood was far too small to be detected by the then available methods. Their success was based on the use of the protein misfolding cyclic amplification (PMCA) technique, which amplified the quantity of PrP^{Sc} more than 10 million-fold, thus raising it to a detectable level. They also found that the quantity of PrP^{Sc}

Table 4 Animal experiments supporting the infectivity of blood in TSE infection

Donor	Recipient	Infectious agent	Inoculation route	Tissues that caused infectivity or showed infectivity/ presence of clinical TSE	Reference
Oral BSE challenged sheep	Sheep	Whole blood	Transfusion	Clinical TSE detected	(Houston et al., 2000)
SE infected mice	Mice	Plasma	Intracerebral	Four out of forty-eight mice developed TSE	(Taylor et al., 2000)
TSE infected mice	Mice	Brain homogenate	Intracerebral	Buffy coat, plasma and fractions IV and V of plasma	(Brown et al., 1999)
Sheep challenged with BSE brain homogenate	Sheep	Blood	Transfusion	Clinical TSE detected	(Siso et al., 2006)
BSE challenged sheep	Sheep	Blood	Transfusion	Clinical TSE detected	(Hunter et al., 2002)

in blood varied among different animals, lending support to the assertion that although blood may be infectious in TSEs, insensitive tests, low concentration levels of the infectious agent in blood, and the type of animal used in previous experiments may all have contributed to the previous failure to demonstrate the presence of the infectious agent in blood.

5.3 Risk of Prion Transmission by Blood

Despite the controversy surrounding the infectivity of blood in TSEs, the use of bovine blood proteins in animal feed appears to pose only minimal risk of TSE transmission based on current scientific evidence and also the expert opinion of various major organizations. In a meeting on 24–26 March 1997 in Geneva, the World Health Organization (WHO), in conjunction with the World Organization for Animal Health (OIE), placed blood and blood products in category IV, representing tissues with no detected infectivity (WHO, 1997). Similarly, at its meeting on 13–14 April 2000, the EU Scientific Steering Committee (SSC) declared that in its opinion, the potential level of infectivity in pooled blood would be minimal provided the blood is sourced from healthy cows and that recommended stunning techniques are used (SSC, 2000). The SSC's recommended approach is validated by Castilla et al. (2005) study, where levels of PrP^{sc} in the blood induced by nonoral exposure to the infectious agent were so low as to be detectable only by 10 million fold amplification of the prions. As the sensitivity of the parenteral route used for this study is typically 10^5 to 10^9 times greater than the oral route for transmission of TSE, depending on the strain of the infectious agent and the genetic disposition of the host (Prusiner et al., 1991), this suggests the levels for oral transmission would be vanishingly small.

In all the animal studies reported so far, prions were only detected in blood when the animals had been exposed to the infectious agent through routes (transfusion or intracerebral) other than the oral route. Where animals were exposed orally to the infectious agent, no PrP^{sc} was found in any of the blood samples (Middleton and Barlow, 1993; Wells et al., 1998; Brown et al., 2000; Espinosa et al., 2007). The only means of exposure to PrP^{sc} through blood proteins in feed is through the oral route. The use of these proteins therefore poses a minimal threat because of the very low sensitivity of the oral route, because oral exposure favors vagal colonization, and lastly because even if blood does harbor PrP^{sc}, it is going to be present at extremely low levels well below the infectious dose.

However, there is still room to further tighten existing preventive measures to eliminate any possibility of exposure to PrP^{sc}. Currently, Japan tests 100% of cattle destined to enter the human food chain for the presence of PrP^{sc} and the EU tests 48%, including all cattle above 30 months of age and every high-risk case. In the US, however, only 1% of cattle are inspected despite the fact that far more cattle are slaughtered in the US than either the EU or Japan (Ackerman and Johnecheck, 2008) and ruminant blood is not banned in ruminant feed. In order to protect American consumers, the US should seriously consider increasing the number of cattle entering the food chain that are tested. Introducing new regulations that make it mandatory for the rendering process to reach the high temperature (133°C) and pressure (3 bars) needed to inactivate TSE agents, as specified by the EU (Directive 94/382/EC), will further ensure that the use of bovine blood proteins as a feed ingredient for US cattle will never lead to any risk of BSE.

6. CONCLUSIONS

The use of whole blood and/or blood-derived proteins as ingredients in animal feed and the human food chain poses only a minimal food safety threat in terms of TSE transmission, exposure to blood allergens and blood-borne pathogens. Any threat posed by their usage would be no different from those due to other foods of animal origin. As efforts to maximize the utilization of these blood proteins as a food and feed ingredient are desirable to make the most of the environmental, economical, and nutritional benefits that the practice has to offer, there is room for both regulators and manufacturers to work together to tighten existing preventive measures, enforce better labeling of products, develop safer blood collection and processing techniques, and educate consumers to allay their largely unjustified concerns regarding these products.

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