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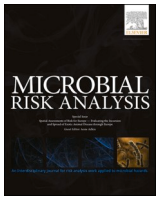

Risk of BSE transmission when fishmeal derived from fish fed bovine spray-dried red blood cells is included in calf milk replacers

C.J. de Vos [a,*], A.F.G. Antonis [a], M.H.J. Sturme [b], M. Appel [b]

[a] Wageningen Bioveterinary Research, Wageningen University & Research, Houtribweg 39, Lelystad, 8221 RA, the Netherlands
[b] Wageningen Food Safety Research, Wageningen University & Research, Akkermaalsbos 2, Wageningen, 6708 WB, the Netherlands

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ABSTRACT

The use of residual streams from agricultural production and food consumption containing animal proteins entails the risk of disease transmission as illustrated by the epidemics of bovine spongiform encephalopathy (BSE) and African swine fever. To combat this risk, the use of animal proteins in livestock feed was banned in the European Union, resulting in a drain of valuable proteins from the agricultural system. With an increasing call for a circular food system, the use of residual streams as a feed ingredient needs to be reconsidered with the associated disease risks being assessed and mitigated where needed. In this study, we assessed the BSE risk of bovine spray-dried red blood cells (SDRBC) as an ingredient of aquafeed. Fish fed with bovine SDRBC could indirectly result in exposure of ruminants to BSE infectivity because one of the exemptions of the feed ban is the use of fishmeal as an ingredient in calf milk replacers. A quantitative risk model was built to evaluate the BSE infectivity present in blood sourced from a slaughtered BSE-infected cow and the reduction of infectivity due to processing steps along the production chain. The end point of the model was the BSE infectivity, expressed in cattle oral ID_{50} ($CoID_{50}$), reaching calves fed calf milk replacer containing fishmeal, and the corresponding probability that this will result in at least one new BSE infection.

The expected BSE infectivity in blood from a BSE-infected cow at the clinical end state of infection is 0.75 $CoID_{50}$ (median value). Infectivity in blood mainly results from cross-contamination with brain tissue during stunning at the slaughterhouse. The initial infectivity is reduced along the pathway from slaughtered cow to calf milk replacer, with the highest reduction achieved by clearance of infectivity by fish fed bovine SDRBC as an ingredient of aquafeed, although this parameter has high uncertainty. The final infectivity reaching calves via inclusion of fishmeal in calf milk replacer is estimated to be very low (median value: 1.1×10^{-5} $CoID_{50}$). Assuming an exponential dose-response model, this corresponds with an expected probability that < 10 out of a million slaughtered BSE-infected cows will result in new BSE infections, which is far below the threshold value of 1 for the basic reproduction number (R0) to initiate a new epidemic. We thus conclude that it is very unlikely that the use of bovine SDRBC as ingredient of aquafeed will result in a new BSE epidemic in cattle. What-if analysis indicated that this conclusion is robust, despite high uncertainty for some input parameters.

Introduction

Agricultural production and food consumption are accompanied by residual streams, only part of which is fed back into the agricultural production system. Current European Union (EU) legislation prohibits the use of specific residual streams containing animal proteins as a feed ingredient because of the risk of disease transmission (EC, 2001, 2009). Feeding of swill (kitchen leftovers and catering waste) has resulted in serious epidemics of contagious animal diseases, such as foot-and-mouth disease in the United Kingdom in 2001 (Davies, 2002), as well as the current African swine fever epidemic that has affected many countries worldwide (Beltran-Alcrudo et al., 2019; Dixon et al., 2019). Similarly, the wide-spread practice of recycling animal proteins into feed for ruminants resulted in a major epidemic of bovine spongiform encephalopathy (BSE) at the end of the 20th century. BSE is caused by a misfolded prion protein (PrP^{SC}) that is extremely heat resistant and was not killed off in the default rendering processes applied at that time (Schreuder et al., 1998; Taylor and Woodgate, 2003). A ban on feeding ruminant proteins in ruminant feed was installed in 1994 to stop transmission of BSE, and in 2001 this was extended to a total ban on

* Corresponding author.
E-mail address: clazien.devos@wur.nl (C.J. de Vos).

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feeding processed animal proteins to livestock (EC, 2001; Ducrot et al., 2010). These bans resulted in a rapid decline of BSE cases in Europe and almost all EU member states now have a negligible risk status for BSE (Arnold et al., 2017; EFSA, 2018; WOAH, 2023a). The extensive surveillance for BSE implemented since 2001 resulted in detection of two additional BSE types in 2004 (Biacabe et al., 2004; Casalone et al., 2004). These atypical BSE types are called H-BSE and L-BSE, because of the respectively higher and lower molecular mass of PrP^{SC} in the Western blot analysis if compared to the classical BSE type (C-BSE) (Alarcon et al., 2023). Atypical BSE occurs sporadically and has only been identified in older bovines (WOAH, 2023a). The origin of the atypical BSE types is unknown, and its risk factors are poorly understood. In recent years, no new C-BSE cases have been detected in the EU, although sporadic cases of atypical BSE are reported in the fallen stock and emergency slaughter surveillance every year (EFSA, 2018, 2022). A few C-BSE cases have, however, been reported by the United Kingdom over the last decade (EFSA, 2022; Scottish Government, 2024).

The European ban on using animal proteins in livestock feed has resulted in an enormous drain of valuable proteins from the agricultural system, which is currently supplemented by plant proteins from external sources, such as import of soy, sunflower and rapeseed. To reduce the ecological footprint of animal production, a circular food system in which residual streams are fed back into the system is key. Concurrently, risks for disease transmission should be assessed and mitigated where needed by, e.g., appropriate treatment of residual streams. Risk assessments of the use of processed animal proteins (PAPs) and ruminant collagen and gelatin by EFSA (EFSA, 2018, 2020) indicated a very low risk given the current BSE epidemiological situation, and in 2021 the use of PAPs derived from poultry in pig feed and the use of PAPs derived from pigs in poultry feed was approved within the EU, as was the use of ruminant collagen and gelatin in feed for non-ruminant animals (EC, 2021).

Blood products derived from slaughtered animals are an equally important source of proteins that could be used as an ingredient in animal feed. In the EU, the use of blood products derived from non-ruminants is allowed for use in feed destined for non-ruminants and fish. Ruminant blood products can, however, not be used in livestock animals and fish because of the possible BSE risk (EC, 2021; Meijer et al., 2023). As a consequence, bovine spray-dried red blood cells (SDRBC) are currently mainly used as an ingredient for human food and pet food, and exported as an ingredient for feed in non-EU countries. SDRBC could, however, also be used as an ingredient of aquafeed and substitute part of the fishmeal currently used in aquafeed (Amer et al., 2022; TMR, 2023). Although this application of SDRBC is common practice outside Europe, it is not allowed in the EU (EC, 2021). Albeit the development of a transmissible spongiform encephalopathy (TSE) infection in fish fed with ruminant proteins is considered extremely unlikely, fish fed with bovine SDRBC could be a hatch of BSE infectivity to ruminants because one of the exemptions of the EU feed ban is the use of fishmeal as an ingredient in calf milk replacer (EC, 2008). As such, the application of SDRBC in aquafeed could thus result in exposure of calves to BSE infectivity if the fishmeal in calf milk replacer is sourced from fish fed bovine SDRBC. This risk was previously assessed by the European Animal Protein Association (EAPA) and EFSA (EFSA, 2007). It was then concluded that a semi-quantitative or quantitative risk assessment was needed to evaluate the risk of bovine SDRBC employed in aquafeed in more detail, but that data to quantify key parameters of such a model were not available. Since then, new experimental results on BSE infectivity in blood of infected cattle have become available (Espinosa et al., 2007; Sohn et al., 2009; Balkema-Buschmann et al., 2021), as well as results from experimental TSE infection studies in fish (Ingrosso et al., 2006; Salta et al., 2009). In addition, the epidemiological situation in Europe has drastically changed with hardly any cases of classical BSE reported over the last decade (EFSA, 2022; WAHIS, 2023b). Furthermore, the need to upgrade and use residual streams in our food production system has become more widely recognized given the limited available environmental resources, resulting in an increased pressure of agriculture on vulnerable ecological systems, and the increasing worldwide demand for animal and plant proteins. However, the need for a circular food system using residual streams to feed animals needs to be balanced against the risks of disease transmission. The objective of this study was to quantitatively assess the risk of new BSE infections when using bovine SDRBC as an ingredient of aquafeed and the subsequent use of fishmeal in calf milk replacer.

Material and methods

Model outline

A quantitative, stochastic risk model was built in Excel and @Risk 8.3.2 (Lumivero, 2023) to estimate the BSE infectivity that could potentially reach calves if bovine SDRBC were allowed as an ingredient in aquafeed, and the corresponding probability that this will result in at least one new BSE infection. To this end, the BSE infectivity present in blood sourced from a single slaughtered BSE-infected cow and the reduction of infectivity due to processing steps along the production chain was evaluated. The BSE infectivity is given as cattle oral ID_{50} ($CoID_{50}$), where 1 $CoID_{50}$ equals the amount of infectivity at which 50 % of the exposed animals will get infected. All steps in the model—from BSE-infected cow to consumption of infectivity by calves—are given in the flowchart of Fig. 1. We assumed collection of bovine blood from a single BSE-infected animal at clinical end-state that escaped on-farm and slaughterhouse detection and is processed as fit for human consumption. Infectivity in the animal's tissues was thus assumed to have reached the clinical infection level. We then followed the hypothetical scenario in which the blood of this animal is processed into SDRBC and subsequently used as an ingredient of aquafeed and fed to fish. The offal of these fish (heads, skeletons, trimmings) is subsequently processed into fishmeal, which is then used as an ingredient in calf milk replacer and fed to calves, resulting in potential exposure of these calves to BSE infectivity. In the model calculations, we assumed a homogenous distribution of BSE infectivity in both the contaminated tissues of an infected animal, and the different products produced along the pathway from slaughtered cow to calf milk replacer (Fig. 1). We did not differentiate between the risk posed by cattle infected with the classical variant of BSE (C-BSE) or the atypical variants (L-BSE and H-BSE).

An overview of all parameters in the model is given in Table 1.

Model calculations

BSE infectivity in bovine blood

The first step in the model is to estimate the BSE infectivity (expressed as $CoID_{50}$) present in blood of an infected animal at clinical end-state. To our knowledge, there are no studies that have been able to detect BSE infectivity in blood. Also, transmission experiments in which blood of infected cattle was used to induce new infections in recipient cattle by intracerebral inoculation or blood transfusion did not result in new infections (EFSA, 2007; Balkema-Buschmann et al., 2021). However, presence of infectivity below the detection limit of the bioassays used cannot be completely ruled out. This limit of detection was estimated to be $10^{-6.4}$ $CoID_{50}$/ml (Wells et al., 2007; Sohn et al., 2009; Konold et al., 2012). A recent experiment of Balkema-Buschmann et al. (2021) in which 24 recipient cows received 0.5 or 1 liter of blood from BSE-infected cows via blood transfusion did not result in any new infections, indicating that infectivity levels in blood might be even below $10^{-9.4}$ $CoID_{50}$/ml (infectivity per liter < 1 intravenous ID_{50}, equaling approximately 0.1 intracerebral ID_{50}, which equals $10^{-6.4}$ $CoID_{50}$) (Brown et al., 1999; Konold et al., 2012; Huang et al., 2020).

BSE infectivity can, however, also result from cross-contamination of blood during slaughter. In Europe, cattle are generally stunned before bleeding using a penetrating captive bolt gun (EFSA, 2020; Pers. comm. L. Heres). This has been shown to introduce fragments of brain tissue

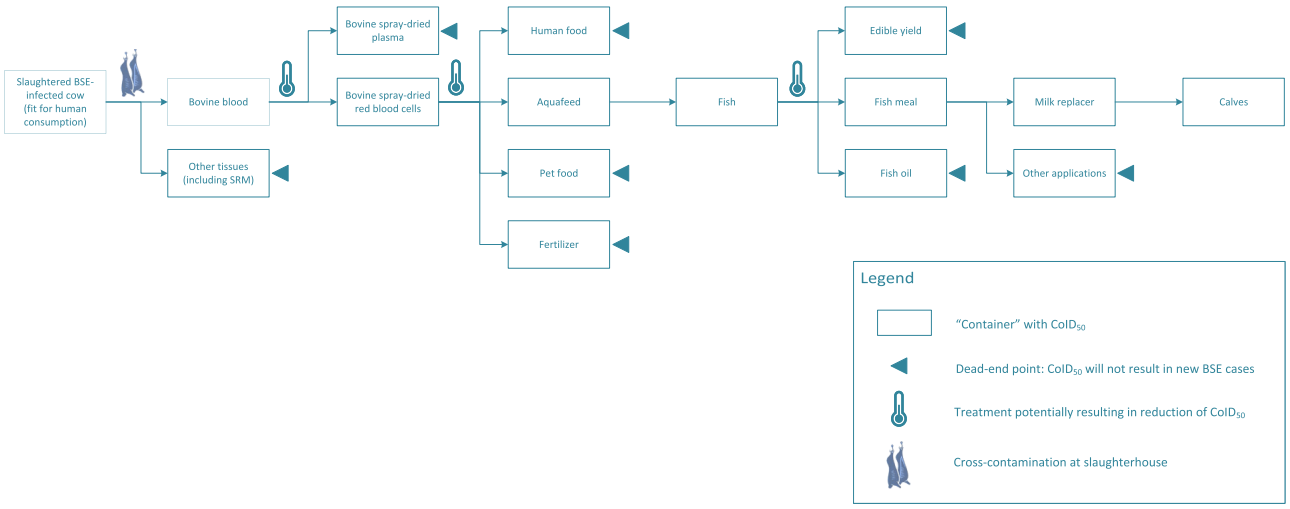

Fig. 1. Flowchart presenting the pathway from a slaughtered BSE infected cow to consumption of BSE infectivity by calves when inclusion of spray-dried red blood cells in aquafeed would be allowed.

into the venous circulation due to brain tissue embolism in a minority of cases. Coore et al. (2005) found brain tissue in blood samples from 3 out of 100 cattle stunned by penetrating captive bolt. Wagner et al. (2019) obtained similar results with 3 out of 103 cattle positive for brain tissue in blood. EFSA (2004) estimated the amount of brain tissue in blood in case of contamination due to embolism at 1.34±0.23 (SE) gram. Although part of the infectivity from brain tissue embolism could be traversed to the heart and lungs or other organs when blood circulation is not stopped quickly after stunning (Ramantanis et al., 2005; EFSA, 2007), we assumed that all infectivity is retained in blood as a worst-case scenario.

Another source of contamination during slaughter would be due to leakage of brain tissue from the captive bolt aperture, resulting in superficial contamination of the skinned cattle head. Troeger (2004) reported contamination of the head with brain tissue in 95 out of 100 examined heads of cattle stunned with penetrative captive bolt. If blood of slaughtered animals is collected directly in a trough, not using a hollow-knife, which is common practice for the production of feed-grade SDRBC, this contamination of the cattle head's surface might partly end up in the blood. There is very little evidence on the amount of brain tissue leaking from the captive bolt aperture. EFSA (2020) estimated that the amount of brain that can spill out during slaughter was between 0.5 to 5 % of total brain tissue, of which only a fraction is likely to contaminate the blood. Further, EFSA (2007) assumed a maximum contamination of blood with brain tissue of 10 mg per liter, irrespective of the mode of contamination. This implies that the amount of contamination due to leakage of brain tissue from the captive bolt aperture is likely to be low compared to contamination due to brain tissue embolism. We assumed that 1 % of spilled out brain will end up in collected blood (Table 1).

The total BSE infectivity present in blood (I_{blood}) in the model is simulated as:

$$I_{blood} = I_{blood_{intr}} + Bernoulli\ (P_{emb}) \times I_{blood_{emb}} + Bernoulli\ (P_{ap}) \times I_{blood_{ap}} \quad (1)$$

Where $I_{blood_{intr}}$ is the estimated intrinsic infectivity in blood, which is assumed to equal the estimated level of detection by bioassays, P_{emb} is the probability that penetrating captive bolt stunning results in contamination of blood due to embolism, $I_{blood_{emb}}$ is the estimated infectivity due to cross-contamination resulting from embolism, P_{ap} is the probability that penetrating captive bolt stunning results in superficial contamination of the cattle's head, and $I_{blood_{ap}}$ is the estimated infectivity due to cross-contamination resulting from leakage from the captive bolt aperture.

I_{blood_intr} is calculated by multiplying the estimated BSE concentration in blood (C_{blood_intr} given in $CoID_{50}$/ml) by the total volume of blood (ml) derived from a slaughtered cow as:

$$I_{blood\_intr} = C_{blood\_intr} \times V_{blood} \times W_{bovine} \quad (2)$$

Where V_{blood} is the volume of blood in an adult bovine per kilogram slaughtered weight and W_{bovine} is the average slaughter weight of a bovine. V_{blood} was set at 60 ml/kg (EFSA, 2007) and W_{bovine} was modelled as a Uniform distribution between 320 and 340 kg, based on the weight of slaughtered bovines in the Netherlands in the period 2020–2022 (CBS, 2023).

$I_{blood_{emb}}$ is calculated by multiplying the amount of brain tissue in blood resulting from embolism (W_{emb}) by the estimated BSE concentration in brain tissue (C_{brain}). Similarly, $I_{blood_{ap}}$ is calculated by multiplying the amount of brain tissue leaking from the captive bolt aperture (W_{ap}) by the estimated BSE concentration in brain tissue (C_{brain}). The concentration of infectivity in brain tissue of animals in the clinical end-state of BSE infection was assumed to have a median value of 6.66 $CoID_{50}$/g (Konold et al., 2012; EFSA, 2020).

BSE infectivity in bovine spray-dried red blood cells (SDRBC)

Citrate anticoagulant is added at blood collection, slightly lowering the pH of blood. This is assumed not to affect the BSE infectivity. To process the blood into SDRBC and spray-dried plasma, the plasma is separated from the cellular fraction (red blood cells and buffy coat); the latter is processed into SDRBC. Since BSE infectivity is strongly associated with PrP^{SC}, which is the result from misfolding of the PrP^{C} protein, it is most likely to be associated with the cellular fraction. We assumed that 83 % of infectivity in blood is present in the cellular fraction (Brown et al., 1999), although there is quite some uncertainty on these values (EFSA, 2007).

After separation of plasma from the cellular fraction, in some production plants the pH of the cellular fraction is increased to 9.8 by adding caustic soda, after which the cellular fraction is spray-dried at a temperature of 80 °C for 30–60 s. This treatment is unlikely to result in any reduction of BSE infectivity (Schreuder et al., 1998; EFSA, 2007).

The total BSE infectivity in SDRBC (I_{SDRBC}) in the model is then calculated as:

$$I_{SDRBC} = I_{blood} \times F_{RBC} \times R_{sd}^{-1} \quad (3)$$

Where F_{RBC} is the fraction of infectivity retained in the cellular

Table 1
Parameters used to model the exposure of calves to BSE infectivity when fish-meal derived from fish fed bovine spray-dried red blood cells is used as an ingredient in calf milk replacer.

Model parameter	Description	Value / Equation	Unit	Source
I_{blood}	Total BSE infectivity in blood	Eq. (1)	$CoID_{50}$	Calculated
$I_{blood_{intr}}$	Intrinsic BSE infectivity in blood	Eq. (2)	$CoID_{50}$	Calculated
$I_{blood_{emb}}$	BSE infectivity in blood resulting from brain tissue embolism due to captive bolt stunning	$W_{emb} \times C_{brain}$	$CoID_{50}$	Calculated
$I_{blood_{ap}}$	BSE infectivity in blood resulting from leakage from captive bolt aperture	$W_{ap} \times C_{brain}$	$CoID_{50}$	Calculated
C_{blood_intr}	BSE concentration in blood	$10^{-6.4}$	$CoID_{50}$/ml	Wells et al., 2007; Konold et al., 2012
C_{brain}	BSE concentration in brain tissue	LognormalAlt(2.5 %=1.25, 50 %= 6.66, 97.5 %= 33.3)[a]	$CoID_{50}$/g	EFSA, 2020
W_{emb}	Weight of brain tissue emboli in blood	Normal (1.34,1.03, Truncate(0))[b]	g	EFSA, 2004
W_{ap}	Weight of brain tissue leakage from captive bolt aperture	$0.01 \times$ Uniform(0.005, 0.05) $\times W_{brain}$	g	Estimate based on EFSA, 2007 and EFSA, 2020
W_{brain}	Weight of brain tissue	475	g	EFSA, 2020
P_{emb}	Probability that captive bolt stunning results in brain tissue embolism in blood	Beta(7,198)	–	Coore et al., 2005; Wagner et al., 2019
P_{ap}	Probability that brain tissue leaks from captive bolt aperture	Beta(96,6)	–	Troeger, 2004
V_{blood}	Volume of blood in bovine	60	ml/kg	EFSA, 2007
W_{bovine}	Slaughter weight of bovine	Uniform(320,340)	kg	CBS, 2023
I_{SDRBC}	BSE infectivity in spray-dried red blood cells	Eq. (3)	$CoID_{50}$	Calculated
F_{RBC}	Fraction of BSE infectivity in red blood cell fraction	Pert(0.5,0.87,1)	–	Brown et al., 1999; EFSA, 2007
R_{sd}	Reduction of BSE infectivity by spray-drying	1	–	EFSA, 2007
$I_{aquafeed}$	BSE infectivity in aquafeed	Eq. (4)	$CoID_{50}$	Calculated
F_{af}	Fraction of produced SDRBC processed into aquafeed	0.8	–	Pers. comm. L. Van Deun,
R_{af}	Reduction of BSE infectivity by extrusion	10^Pert (0.1,0.2,1.0)	–	Schreuder et al., 1998; Sørensen, 2012
I_{fish}	BSE infectivity in fish	Eq. (5)	$CoID_{50}$	Calculated
P_{GI}	Probability that BSE infectivity is retained in gastro-intestinal tract of fish	Beta(s + 1,n - s + 1)[c]	–	Estimate based on Ingrosso et al., 2006
T_{GI}	Time period during which infectivity can be recovered from the gastro-intestinal tract of fish	Discrete Uniform (1,15,30,60,90)	day	Estimate based on Ingrosso et al., 2006
T_{fast}	Length of fasting period before harvesting fish	Pert(1,1,2)	day	Alltech Coppens, 2023
T_{rear}	Rearing period of fish	365	day	Alltech Coppens, 2023
$I_{fishmeal}$	BSE infectivity in fishmeal	Eq. (6)	$CoID_{50}$	Calculated
F_{bp}	Fraction of farmed fish that is byproducts	0.45	–	Aspevik et al., 2017
F_{fm}	Fraction of BSE infectivity of fish byproducts in fishmeal	1	–	Assumption that all infectivity is associated with the protein fraction
R_{fm}	Reduction of BSE infectivity when processing fish byproducts into fishmeal	1	–	FAO, 1986; Schreuder et al., 1998
I_{CMR}	BSE infectivity in calf milk replacer	Eq. (7)	$CoID_{50}$	Calculated
F_{CMR}	Fraction of produced fishmeal processed into calf milk replacer	0.05	–	Assumption
P_{BSE}	Probability ≥ 1 new BSE infections	Eq. (8)	–	Calculated
N_{BSE_und}	Annual number of undetected BSE-infected cattle slaughtered in the EU	PertAlt(2.5 %= 3.61, 50 %= 11.38, 97.5 %= 19.79)[d]	–	EFSA, 2018

[a] Lognormal distribution with median value of 6.66 and 95 % confidence interval between 1.25 and 33.3.
[b] Left-truncated normal distribution of which the minimum value sampled is 0.
[c] The values of s (number of fish tested positive) and n (total number of fish tested) were dependent on the value of T_{GI} and based on results for rainbow trout only. If $T_{GI} = 1$: $s = 1$ and $n = 8$; If $T_{GI} = 15$: $s = 0$; $n = 8$; If $T_{GI} = 30$: $s = 0$, $n = 8$; If $T_{GI} = 60$: $s = 0$, $n = 6$; If $T_{GI} = 90$: $s = 0$, $n = 11$. Furthermore, the sampled value of P_{GI} at $T_{GI} = i$ was set conditional on the value of P_{GI} at $T_{GI} = i - 1$ to ensure a decreasing probability over time, i.e. $P_{GI} = Min\left(P_{GI\,T_{GI}=i}, P_{GI\,T_{GI}=i-1}\right)$.
[d] Pert distribution with median value of 11.38 and 95 % confidence interval between 3.61 and 19.79.

fraction, and R_{sd} is the reduction of infectivity achieved by the spray-drying process (here set to 1 as we assumed no reduction at all).

BSE infectivity in aquafeed

We assumed production of aquafeed by extrusion, which requires higher levels of moisture, temperature and pressure than pelletization

(Lundblad et al., 2011). Extrusion is based on a minimum temperature of 100 °C for a short period (< 2 min) under high pressure (20–30 bar) (Sørensen, 2012). No information is available on the effectivity of this treatment to reduce BSE infectivity. Based on a comparison of the extruding conditions to the treatments evaluated by Schreuder et al. (1998), we assumed that if extrusion is done at a minimum temperature of 120 °C and under high pressure, a slight inactivation of infectivity will be achieved with an expected reduction factor of 0.2 $\log_{10}$.

Currently, inclusion of bovine SDRBC as an ingredient in aquafeed is not allowed (EC, 2001). The fraction of SDRBC that will be processed into aquafeed if legally permitted is not known and will depend on multiple factors, such as the availability and price of other ingredients for aquafeed, alternative markets for SDRBC such as human food, pet food and fertilizer, and the maximum inclusion rate in aquafeed for optimal fish production. Currently, approximately 20 % of bovine SDRBC is used as an ingredient for human food and pet food. The remaining 80 % would be available for inclusion in aquafeed (Pers. comm. L. Van Deun). In the default model calculations, we assumed that 80 % of SDRBC is used for aquafeed production.

The total infectivity in aquafeed ($I_{aquafeed}$) in the model is then calculated as:

$$I_{aquafeed} = F_{af} \times I_{SDRBC} \times R_{af}{}^{-1} \quad (4)$$

Where F_{af} is the estimated fraction of produced SDRBC that is processed into aquafeed, and R_{af} is the reduction of infectivity achieved by the extrusion process in the production of aquafeed.

BSE infectivity in fish

There is currently no evidence that fish are susceptible to TSE infection. The species barrier between cattle and fish is probably high, based on the low degree of PrP^C gene sequence homology, increasing the infectious dose needed for infection and therewith decreasing the likelihood of infection upon exposure (Matthews and Cooke, 2003). Several experiments have been conducted to investigate if inoculation of fish with TSE infectivity (BSE or scrapie) could result in infection (SSC, 2003; Ingrosso et al., 2006; Salta et al., 2009). None of these studies have been able to demonstrate infection, although some abnormal brain pathology was observed in gilthead sea bream (*Sparus aurata*) in the study of Salta et al. (2009). Results of the western blot analysis for PrP^{SC} were, however, negative in this study, indicating absence of prion disease. Ingrosso et al. (2006) tested several tissues of turbot (*Scophthalmus maximus*) and rainbow trout (*Oncorhynchus mykiss*) at 1, 15, 30, 60 and 90 days after forced feeding of scrapie infectivity. In 1 out of 8 challenged trouts, infectivity could be recovered from the intestines 1 day after infection; none of the challenged turbots had remaining infectivity in the intestines. Results from this experiment indicate that TSE infectivity is probably quickly cleared from fish tissue. The observation period of 90 days was, however, relatively short and it cannot completely be ruled out that infection might establish at a later time point (Ingrosso et al., 2006). Based on the evidence available, we assumed that BSE infectivity in aquafeed has a low probability to be retained in the gastro-intestinal tract of fish for a limited period of time, with the probability of retention decreasing by time. The probabilities and time periods used were based on the observations of Ingrosso et al. (2006) for scrapie in rainbow trout. Also, we considered a one- to two-day fasting period before harvesting (Alltech Coppens, 2023), which will reduce the amount of remaining BSE infectivity in the gastro-intestinal tract.

The total BSE infectivity in fish (I_{fish}) in the model is calculated as:

$$I_{fish} = P_{GI} \times I_{aquafeed} \times \frac{(T_{GI} - T_{fast})}{T_{rear}} \quad (5)$$

Where P_{GI} is the probability that BSE infectivity is retained in the gastro-intestinal tract of fish, T_{GI} is the time period during which infectivity can be recovered from the gastro-intestinal tract, T_{fast} is the length of the fasting period before harvesting, and T_{rear} is the rearing period of fish. The estimates for T_{GI}, T_{fast}, and T_{rear} were all based on data for rainbow trout. The rearing period of trout depends on the water temperature and ranges from 8 to 9 months under optimal conditions, but can be 12 to 18 months for outside farming (Alltech Coppens, 2023).

BSE infectivity in fishmeal

Only the byproducts of farmed fish will be processed into fishmeal. The percentage of byproducts differs per fish species (Newton and Little, 2013). We assumed an average of 45 % byproducts based on figures for total global fish production (Aspevik et al., 2017). Fishmeal is made by cooking, pressing, drying and grinding of fish or fish waste, resulting in a 20–25 % yield (Windsor, 2001; Tacon and Metian, 2008). The materials lost are mainly water and some fat, whereas proteins are largely retained (Windsor, 2001). Since BSE infectivity is associated with the PrP^{SC} protein, we assumed that all infectivity present in fish byproducts would be retained in fishmeal. Heat treatment during fishmeal production ("cooking" at 95–100 °C for 15–20 min, and drying at 95 °C) (FAO, 1986) is not expected to result in a reduction of BSE infectivity (Schreuder et al., 1998).

The total BSE infectivity in fishmeal ($I_{fishmeal}$) in the model is calculated as:

$$I_{fishmeal} = I_{fish} \times F_{bp} \times F_{fm} \times R_{fm}{}^{-1} \quad (6)$$

Where F_{bp} is the fraction of farmed fish that is considered byproducts (not used for human consumption), F_{fm} is the fraction of BSE infectivity of fish byproducts that is expected to end up in fishmeal, and R_{fm} is the reduction of BSE infectivity achieved by processing fish byproducts into fishmeal (here set to 1 as we assumed no reduction at all).

BSE infectivity in calf milk replacer

Inclusion of fishmeal as an ingredient in calf milk replacer does not change the BSE infectivity present, i.e., if all fishmeal would be processed into calf milk replacer, there is no loss of infectivity in this step of the production chain. The use of fishmeal in calf milk replacer is, however, limited due to economic and quality constraints. Prices of fishmeal are currently too high to make its use in calf milk replacer economically attractive and the resulting product is smelly and easily spoilt due to the remaining oil fraction in fishmeal (Pers. comm. P. Mölder). Therefore, we assumed that 5 % of fishmeal production could be processed in calf milk replacer.

The total BSE infectivity in calf milk replacer (I_{CMR}) is calculated as:

$$I_{CMR} = F_{CMR} \times I_{fishmeal} \quad (7)$$

Where F_{CMR} is the estimated fraction of produced fishmeal that is used as ingredient in calf milk replacer.

Probability of new BSE infections

The probability that ingestion of BSE infectivity by calves will result in one or more new BSE infections (P_{BSE}) is then calculated using an exponential distribution, assuming that there is no threshold dose for prions (Gale, 1998):

$$P_{BSE} = 1 - exp^{-(ln\,(2)\ \times I_{CMR})} \quad (8)$$

Similarly, the hypothetical probability of one or more new BSE infections if intermediate products along the production chain were ingested by calves was calculated by substituting I_{CMR} in Eq. (8) by I_{blood}, I_{SDRBC}, $I_{aquafeed}$, I_{fish}, and $I_{fishmeal}$, respectively.

Annual exposure to BSE infectivity in the EU

To estimate the EU-wide exposure of calves to BSE infectivity when including fishmeal derived from fish fed SDRBC is included in calf milk replacers, I_{CMR} was multiplied by the annual number of BSE-infected cattle that is expected to be slaughtered undetected in the EU (N_{BSE_und}). EFSA (2018) estimated N_{BSE_und} to be 11.4 animals per year.

Model output

Main output parameters of the risk model are I_{CMR}, i.e. the BSE infectivity in calf milk replacers that will be ingested by calves, and P_{BSE}, which is the probability that this infectivity will result in one or more new BSE infections. Model results are based on 10,000 iterations to account for uncertainty in model input parameters.

Uncertainty analysis

To evaluate the impact of uncertain input parameters on model output, correlation coefficients between these input parameters and the main output parameters were calculated in @Risk. This sensitivity analysis could only be performed for uncertain input parameters that were modelled by a probability distribution. To explore the impact of other input parameters and assumptions made, several what-if scenarios were run with the model. An overview of all what-if scenarios is given in

Table 2
Overview of what-if scenarios explored with the risk model.

Scenario	Uncertainty addressed	Description	Parameters changed	Rationale
WI-1	BSE infectivity in blood	Lower intrinsic infectivity in blood	$C_{blood\_intr} = 10^{-9.4}$ $CoID_{50}$/ml	Balkema-Buschmann et al., 2021
WI-2	BSE infectivity in blood	Higher level of cross-contamination of blood at slaughter	$W_{emb} = 10$ g; $W_{ap} = 0.1 \times \text{Uniform}(0.005, 0.05) \times W_{brain}$	EFSA 2004; EFSA 2007
WI-3	BSE infectivity in blood	BSE-infected animal slaughtered 1 month before clinical end-state	$C_{blood\_intr} = 10^{-6.4} \times 2^{-(T_{dt}^{-1})}$ $CoID_{50}$/ml; $C_{brain} = \text{LognormalAlt}(2.5\ \% = 1.25,\ 50\ \% = 6.66,\ 97.5\ \% = 33.3) \times 2^{-(T_{dt}^{-1})}$ $CoID_{50}$/g [a]	Assumption
WI-4	BSE infectivity in blood	Infectivity in blood homogenously distributed over red blood cell fraction and plasma	$F_{RBC} = 0.4$	Assumption
WI-5	Accumulation of BSE infectivity in fish	Jeffrey's prior to estimate beta distribution for P_{GI}	$P_{GI} = \text{Beta}(s + 0.5, n - s + 0.5)$ [b]	Ingrosso et al., 2006
WI-6	Accumulation of BSE infectivity in fish	Lower probability of infection retained in gastro-intestinal tract of fish	$P_{GI} = \text{Beta}(s + 1, n - s + 1)$ [c]	Ingrosso et al., 2006
WI-7	Accumulation of BSE infectivity in fish	All infectivity retained in gastro-intestinal tract of fish	$P_{GI} = 1; \frac{(T_{GI} - T_{fast})}{T_{rear}} = 1$	Worst-case assumption
WI-8	Accumulation of BSE infectivity in fish	All infectivity in fish processed into fishmeal	$F_{bp} = 1$	Assumption infectivity only present in byproducts
WI-9	Inactivation of BSE by the production of aquafeed	No inactivation when producing aquafeed	$R_{af} = 0$	Assuming temperatures reached during extrusion will not result in inactivation at all
WI-10	Inactivation of BSE by the production of aquafeed	Higher inactivation when producing aquafeed	$R_{af} = \text{Pert}(2.1, 2.2, 2.5)$	Extrusion as effective as method C of Schreuder et al., 1998
WI-11	Destination of ingredients along the infection pathway	Smaller fraction (10 %) of SDRBC processed into aquafeed	$F_{af} = 0.1$	Assumption
WI-12	Destination of ingredients along the infection pathway	All SDRBC processed into aquafeed	$F_{af} = 1$	Assumption
WI-13	Destination of ingredients along the infection pathway	Higher fraction (25 %) of fishmeal used as ingredient in calf milk replacer	$F_{CMR} = 0.25$	Assumption
WI-14	Destination of ingredients along the infection pathway	All bovine SDRBC processed into aquafeed and all fishmeal processed into calf milk replacer	$F_{af} = 1$; $F_{CMR} = 1$	Worst-case assumption

[a] T_{dt} = infectivity doubling time (months) in subclinical animals, modelled as Pert(0.95,1.2,2.4) (EFSA, 2018).

[b] The values of s (number of fish tested positive) and n (total number of fish tested) were dependent on the value of T_{GI} and based on results for rainbow trout only. If $T_{GI} = 1$: $s = 1$ and $n = 8$; If $T_{GI} = 15$: $s = 0$; $n = 8$; If $T_{GI} = 30$: $s = 0$, $n = 8$; If $T_{GI} = 60$: $s = 0$, $n = 6$; If $T_{GI} = 90$: $s = 0$, $n = 11$. Furthermore, the sampled value of P_{GI} at $T_{GI} = i$ was set conditional on the value of P_{GI} at $T_{GI} = i - 1$ to ensure a decreasing probability over time, i.e. $P_{GI} = Min\left(P_{GI\,T_{GI}=i}, P_{GI\,T_{GI}=i-1}\right)$.

[c] The values of s (number of fish tested positive) and n (total number of fish tested) were dependent on the value of T_{GI} and based on combined results for rainbow trout and turbot. If $T_{GI} = 1$: $s = 1$ and $n = 16$; If $T_{GI} = 15$: $s = 0$; $n = 16$; If $T_{GI} = 30$: $s = 0$, $n = 16$; If $T_{GI} = 60$: $s = 0$, $n = 12$; If $T_{GI} = 90$: $s = 0$, $n = 19$ (Ingrosso et al., 2006). Furthermore, the sampled value of P_{GI} at $T_{GI} = i$ was set conditional on the value of P_{GI} at $T_{GI} = i - 1$ to ensure a decreasing probability over time, i.e. $P_{GI} = Min\left(P_{GI\,T_{GI}=i}, P_{GI\,T_{GI}=i-1}\right)$.

Table 2.

The uncertainty on BSE infectivity in blood was challenged in four what-if scenarios. In scenario WI-1, we assumed a lower intrinsic infectivity in blood, based on the recent findings by Balkema--Buschmann et al. (2021) who did not find any new infections, not even when a liter of blood was transfused. In scenario WI-2, we assumed a higher cross-contamination of blood at slaughter, based on figures given by EFSA (2004; 2007) as a worst-case scenario. In scenario WI-3, we assumed that the cow was slaughtered one month before clinical onset of disease, and therefore had a lower level of BSE infectivity in all tissues at the moment of slaughter. In scenario WI-4, we assumed that infectivity in blood is homogenously distributed over the cellular fraction (40 % of blood volume) and the plasma fraction (60 % of blood volume).

The uncertainty on accumulation of BSE infectivity in fish was challenged in another four what-if scenarios. In scenario WI-5, we used Jeffrey's prior rather than an uninformed (uniform) prior to estimate the probability that infectivity is retained in the gastro-intestinal tract. In scenario WI-6, we assumed a lower probability that infectivity is retained in the gastro-intestinal tract, by combining the results of Ingrosso et al. (2006) for rainbow trout and turbot. In scenario WI-7, we assumed that all infectivity would be retained in the gastro-intestinal tract, i.e. no dilution of infectivity in this step of the production chain. This is a very unlikely worst-case scenario. In scenario WI-8, we assumed that all infectivity retained in the gastro-intestinal tract of fish would end up in fish byproducts and be processed into fishmeal.

The uncertainty on inactivation of BSE by the production of aquafeed (extrusion), was challenged in two more what-if scenarios. In scenario WI-9, we assumed no inactivation at all, whereas in scenario WI-10, we assumed inactivation by extrusion to be as efficient as heat treatment at 125 °C for 15 min (method C described by Schreuder et al., 1998).

The uncertainty on the destination of the different ingredients along the production chain was challenged in the last four what-if scenarios. In scenario WI-11, we assumed that only 10 % of SDRBC would be processed into aquafeed, whereas in scenario WI-12, we assumed that all SDRBC (100 %) would be processed into aquafeed. In scenario WI-13, we assumed that 25 % of fishmeal would be processed into calf milk replacer. In scenario WI-14, we assumed a worst-case scenario in which all SDRBC would be processed into aquafeed, and all fishmeal would be processed into calf milk replacer.

Results

In the baseline scenario, the expected median infectivity in calf milk replacer to which calves are exposed (I_{CMR}) is 1.1×10^{-5} $CoID_{50}$ (95 % uncertainty interval: $6.3 \times 10^{-8} - 3.0 \times 10^{-4}$). The expected median probability of a new BSE infection (P_{BSE}) is 7.3×10^{-6} (95 % uncertainty interval: $4.4 \times 10^{-8} - 2.0 \times 10^{-4}$). The expected median infectivity in blood collected at the slaughterhouse (I_{blood}) is 0.75 $CoID_{50}$ (95 % uncertainty interval: $7.8 \times 10^{-3} - 8.6$), resulting in P_{BSE} is 0.41 (95 % uncertainty interval: $5.4 \times 10^{-3} - 0.997$) if no inactivation would occur down the production chain. Accounting for the annual number of undetected BSE-infected cattle in the EU, the median overall exposure of calves in the EU is estimated at 1.1×10^{-4} $CoID_{50}$ per year (95 % uncertainty interval $6.6 \times 10^{-7} - 3.7 \times 10^{-3}$). The wide uncertainty intervals are primarily caused by the uncertainty on whether or not blood is cross-contaminated by brain tissue resulting from embolism and/or leakage from the captive bolt aperture. The majority of BSE infectivity in blood results from cross-contamination. The biggest reduction in infectivity is made in the step from aquafeed to fish (Fig. 2).

Model results were most sensitive to the uncertainty on input parameters contributing to the BSE infectivity in blood of slaughtered cows, i.e., the BSE concentration in brain tissue (C_{brain}), the probability that brain tissue leaks from captive bolt aperture (P_{ap}), the weight of brain tissue leakage from captive bolt aperture (W_{ap}), and the probability that captive bolt stunning results in brain tissue embolism in blood (P_{emb}). Also uncertainty on the estimated BSE infectivity in fish at harvesting, based on the time-dependent probability that BSE infectivity is retained in gastro-intestinal tract of fish (P_{GI}) and the time period during which infectivity can be recovered from the gastro-intestinal tract (T_{GI}), had quite some impact on model results. Uncertainty on the reduction of BSE infectivity by extrusion when producing aquafeed (R_{af}) also impacted model results to some extent. Model results were most sensitive to values sampled from the tails of the uncertainty distributions of these input parameters (Fig. 3).

The what-if scenarios provided more insight into the impact of model assumptions on model results. Scenario WI-7, in which we assumed that all infectivity ingested by fish will still be present at harvest, resulted in the highest risk estimate with a median expected infectivity ingested by calves (I_{CMR}) of 5.4×10^{-3} $CoID_{50}$ (95 % uncertainty interval: $5.6 \times 10^{-5} - 6.6 \times 10^{-2}$) and a median probability of a new BSE infection (P_{BSE}) of 3.7×10^{-3} (95 % uncertainty interval: $3.9 \times 10^{-5} - 4.5 \times$

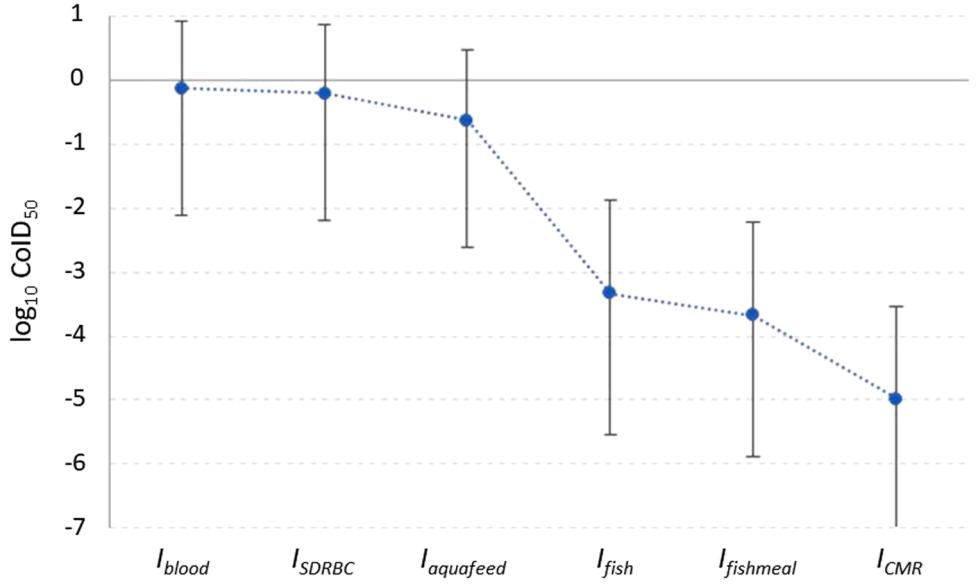

Fig. 2. Expected BSE infectivity levels (median $\log_{10}$ $CoID_{50}$ and 95 % uncertainty interval) in blood, spray-dried red blood cells (SDRBC), aquafeed, fish, fishmeal and calf milk replacer (CMR) in the default scenario.

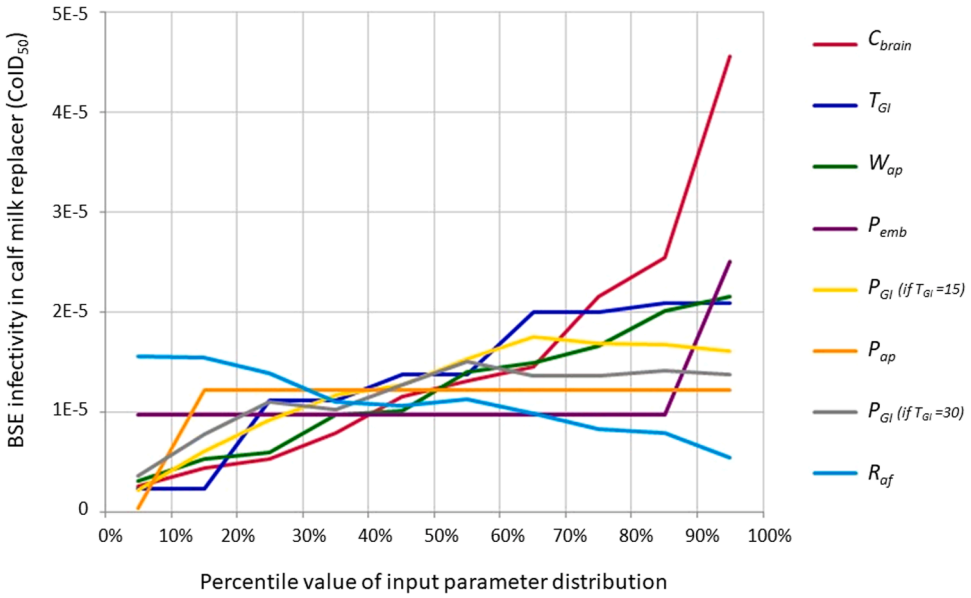

Fig. 3. Sensitivity of the estimated BSE infectivity in calf milk replacer (I_{CMR}) to the most important uncertain input parameters. The lines indicate the change in the median value of I_{CMR} across the range of input values sampled for each of the individual input parameters. C_{brain} = BSE concentration in brain tissue; T_{GI} = Time period during which infectivity can be recovered from the gastro-intestinal tract of fish; W_{ap} = Weight of brain tissue leakage from captive bolt aperture; P_{emb} = Probability that captive bolt stunning results in brain tissue embolism in blood; P_{GI} = Probability that BSE infectivity is retained in the gastro-intestinal tract of fish; P_{ap} = Probability that brain tissue leaks from captive bolt aperture; R_{af} = Reduction of BSE infectivity by extrusion.

10^{-2}). This risk is approximately 500 times higher than the risk of the baseline scenario (Fig. 4). Nevertheless, model results thus indicate that even in this extremely unlikely scenario, the risk of new BSE infections is very low. Assumptions on the use of SDRBC in aquafeed and the use of fishmeal in calf milk replacer had a relatively high impact on model results compared to most other uncertainties. Scenario WI-14, in which we assumed that all contaminated bovine SDRBC is processed into aquafeed AND that all fishmeal derived from fish fed with this aquafeed is used as an ingredient in calf milk replacer, resulted in an elevated risk which is approximately 25 times higher than the baseline scenario. Uncertainty on inactivation of BSE by the production of aquafeed also impacted model results as shown by scenario WI-10, which indicates that a higher inactivation by extrusion could result in a 100-fold lower risk compared to the baseline scenario. Assumptions on the infectivity in blood had less impact on model results, although a higher level of cross-contamination at slaughter (WI-2) resulted in a 10-fold higher risk than in the baseline scenario. All other scenarios resulted in only a slight increase or decrease of the risk (Fig. 4).

Discussion

The estimated risk of new BSE infections when including fishmeal in calf milk replacer that is derived from fish fed bovine SDRBC is estimated to be very low with an expected probability that < 10 out of a million BSE-infected cows slaughtered at clinical end state would result in one or more new BSE infections if the animals were declared fit for human consumption. To our knowledge, BSE infectivity has never been demonstrated in blood of BSE-infected cattle, in contrast to, e.g., blood derived from sheep infected with BSE or scrapie, or from humans infected with Creutzfeldt Jacob disease (EFSA, 2007; Espinosa et al., 2007; Kumagai et al., 2019; Balkema-Buschmann et al., 2021; Pozzo di Borgo et al., 2023). In the model calculations, an intrinsic infectivity in blood just below the threshold level of BSE detection in bioassays (mouse) was assumed as a conservative (worst-case) approach (Konold et al., 2012; EFSA, 2020). Based on a recent experiment by Balkema--Buschmann et al. (2021) in which 0.5 or 1 liter blood of 12 BSE-infected donor animals (6 C-BSE, 3 H-BSE, and 3 L-BSE) was transfused to 24 recipient animals, none of which developed BSE over a period of 10 years, it is likely that the infectivity levels in blood are even 2.7 – 3 $\log_{10}$ lower, if infectivity is present at all. We explored the effect of a lower intrinsic infectivity in the what-if analysis (WI-1), but this did not change our risk estimate (Fig. 4). This is explained from the fact that the estimated levels of BSE infectivity in blood in the risk model mainly result from cross-contamination at slaughter rather than from the intrinsic infectivity. In Europe, most slaughterhouses use penetrating captive bolt to stun cattle before bleeding (EFSA, 2020; Pers. comm. L. Heres), which might result in brain tissue embolism introducing fragments of brain tissue into the venous circulation (Coore et al., 2005; Wagner et al., 2019). Also, the collected blood may be contaminated due to leakage of brain tissue from the captive bolt aperture when feed-grade blood is collected in a trough (Troeger, 2004). Although some quantitative data were available on the probability and amount of brain tissue embolism due to stunning, we had very little information on the possible leakage of brain tissue from the captive bolt aperture. Therefore, we challenged our assumptions on cross-contamination of blood with brain tissue in the what-if analysis (WI-2) and this resulted in a 10-fold higher risk (Fig. 4). Our worst-case approach by assuming that a BSE-infected cow at clinical end state would be processed as fit for human consumption, resulted in a slight overestimate of the risk. We challenged this assumption in what-if analysis (WI-3), where we based the risk on the infectivity level in a cow that is slaughtered one month before reaching the clinical end stated. This resulted in a 40 % reduction of the risk (Fig. 4).

The total infectivity in blood (I_{blood}) of a BSE-infected cow at clinical end state was estimated to be <1 $CoID_{50}$ (Fig. 2), indicating that even if no reduction of infectivity would be achieved down the production chain (see Fig. 1), the probability of a new epidemic due to feeding of calf milk replacer including fishmeal derived from fish fed bovine SDRBC is low. Inclusion of bovine SDRBC in aquafeed can only result in a new BSE epidemic if the infectivity in calf milk replacer fed to calves would on average result in more than one new BSE infection, i.e. if the basic reproduction number (R0) is above 1. The probability that ingestion of 1 $CoID_{50}$ results in infection is 50 %, i.e., on average only 1 out of 2 cattle receiving 1 $CoID_{50}$ will develop BSE, which equals an R0 value of 0.5. With a median value of 0.75 $CoID_{50}$ in blood sourced from a BSE-infected cow, the probability of at least one new infection is only 0.41

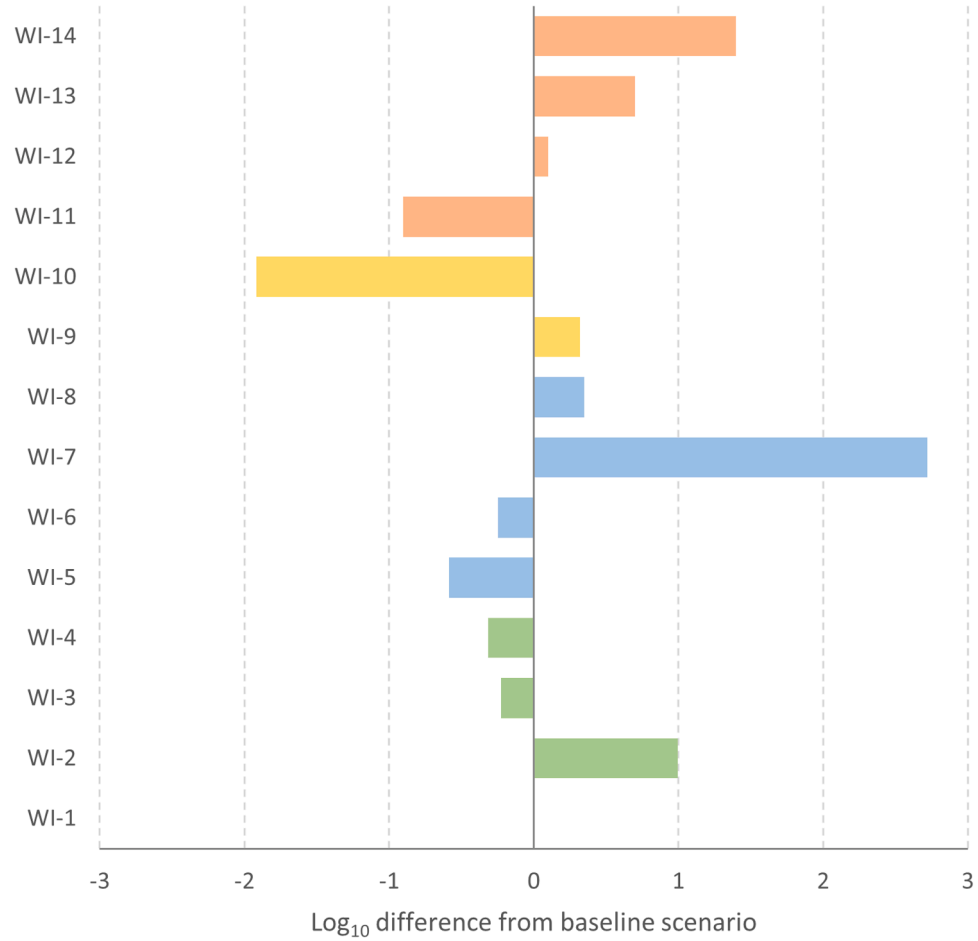

Fig. 4. Relative increase or decrease (expressed as $\log_{10}$ difference) of the BSE infectivity in calf milk replacer (I_{CMR}) compared to the baseline scenario for 13 what-if scenarios. Green: scenarios addressing uncertainty on BSE infectivity in blood; blue: scenarios addressing uncertainty on accumulation of BSE infectivity in fish; yellow: scenarios addressing uncertainty on inactivation of BSE by the production of aquafeed; orange: scenarios addressing uncertainty on the destination of the different ingredients along the infection pathway. A more detailed description of each scenario is given in Table 2.

if this blood is directly fed to calves. However, most steps of the production chain presented in Fig. 1 result in reduction of the BSE infectivity that proceeds to the next step, reducing the probability of at least one new infection to 7.3×10^{-6}. Even in the worst-case scenario where all infectivity in fish is retained in the production chain (WI-7), the probability of at least one new infection is only 3.7×10^{-3}. Based on these results, we conclude that it is very unlikely that inclusion of bovine SDRBC in aquafeed will result in a new BSE epidemic in cattle. This conclusion is in agreement with the fact that BSE prevalence levels in countries that do allow the use of bovine blood products in aquafeed, such as the USA (FDA, 2008), have been maintained at very low levels throughout time (WOAH, 2023a).

The risk estimate presented above is based on the blood of a single BSE-infected cow. To estimate the total exposure of calves in Europe via BSE infectivity in calf milk replacer, the estimated infectivity in calf milk replacer (I_{CMR}) should be combined with the expected number of BSE-infected cows being slaughtered undetected. The number of BSE-infected cows that is annually detected in the EU by the different surveillance components has decreased tremendously over the last decade and was <10 per year in the period 2015–2021 (WOAH, 2023b; EFSA, 2022). The majority of these infections were typed as H-BSE and L-BSE; no C-BSE infections were detected in the EU since 2019, although the United Kingdom reported on an additional two C-BSE cases in 2021 and 2024 (Scottish Government, 2024). Based on 2015 surveillance data, the number of BSE-infected cattle (C-BSE, H-BSE and L-BSE combined) that is slaughtered undetected each year in the EU was estimated at 11.4 (95 % uncertainty interval 3.6 – 19.8) (EFSA, 2018). Given the low, but stable level of BSE infections detected in the EU during the period 2015–2021, we considered this estimate representative for the current situation. If blood sourced from all undetected cattle would be used in aquafeed, the median infectivity ending up with calves would be approximately 1.1×10^{-4} $CoID_{50}$ per year (95 % uncertainty interval $6.6 \times 10^{-7} – 3.7 \times 10^{-3}$). This still implies a very low risk of new BSE infections and is below the estimated BSE exposure of cattle due to possible contamination of ruminant feed with BSE-infected processed animal protein (PAP) after authorizing pig PAP in poultry feed and poultry PAP in pig feed (EFSA, 2020).

Model calculations did not account for contamination of ruminant feed via cross-contamination along the production chain. Cross-contamination of ruminant feed with aquafeed containing bovine SDRBC was considered extremely unlikely as aquafeed is only produced in dedicated feed mills in Europe, i.e., aquafeed production is physically separated from feed production for ruminants, pigs and poultry (EFSA, 2020). Similarly, cross-contamination of ruminant feed with aquafeed containing bovine SDRBC at the farm level is deemed impossible, as mixed farming of ruminants and fish is not present. At fishmeal production level, contamination could also occur if production facilities or transportation means were shared with the production processes for PAP of other animal species. However, legal requirements for production of fishmeal that is used in calf milk replacer prescribe production in processing plants dedicated exclusively to production of fish-derived products (EC, 2008). Lastly, in the unlikely event that

cross-contamination of ruminant feed with fishmeal would occur, this would concern low amounts of infectivity compared to direct inclusion of fishmeal in calf milk replacer (EFSA, 2018). Also, adult cattle are not likely to be infected by oral ingestion of BSE infectivity, with the susceptibility to BSE infection being highest in young animals (up to an age of 6 months) (Arnold and Wilesmith, 2004). Similarly, adult cattle could be accidentally exposed to contaminated fishmeal in calf milk replacer at the farm level, which again entails a lower risk than the direct feeding of this fishmeal to calves.

Main uncertainties in the model were related to the retention of BSE infectivity in the gastro-intestinal tract of fish (P_{GI} and T_{GI}), and the inactivation of BSE by the production of aquafeed (extrusion) (R_{af}). Prions are extremely heat resistant and even rendering at 133 °C at 3 bar for at least 20 min does not fully inactivate BSE infectivity (Schreuder et al., 1998; EFSA, 2005). Extrusion is also done at high temperatures (120–130 °C) and under high pressure (20–30 bar), but for a much shorter time period (< 2 min) (Sørensen, 2012). No data were available to directly estimate the inactivation by extrusion. Our model input parameter was based on a comparison of the extrusion conditions to the different heating conditions tested by Schreuder et al. (1998). We explored the effect of this assumption in the what-if analysis. If extrusion would not result in any inactivation of BSE (WI-9), the risk is two times higher, whereas if extrusion would be more effective (WI-10), the risk is 100-fold lower.

Very little data were available on the fate of BSE infectivity in fish. In an experiment with rainbow trout (*Oncorhynchus mykiss*) and turbot (*Scophthalmus maximus*), the majority of fish fed with a mouse-adapted scrapie-strain cleared the infectivity within one day (Ingrosso et al., 2006). No TSE infection of fish was observed after challenge to scrapie or BSE via oral or parenteral routes (Ingrosso et al., 2006; Salta et al., 2009), although the period of observation by Ingrosso et al. (2006) might have been too short to observe infection (Friedland et al., 2009). The estimates in our model for the probability that BSE infectivity is retained in the gastro-intestinal tract of fish (P_{GI}) were based on observations by Ingrosso et al. (2006) at different time points after inoculation and had a wide uncertainty interval due to the limited number of observations (Table 1). This left our results sensitive to the selected prior for the beta distribution to model P_{GI}; using Jeffrey's prior rather than an uniformed prior resulted in a 4-fold reduction of the estimated risk (WI-5). Both the sensitivity analysis (Fig. 3) and the what-if analysis (Fig. 4) illustrated that model results were sensitive to the values used to model the length of time (T_{GI}) and the probability (P_{GI}) of BSE infectivity retention in the gastrointestinal tract of fish. In the unlikely event that all infectivity would be retained in the gastro-intestinal tract of fish, the infection risk is increased 500-fold (WI-7), whereas a lower probability that infection is retained in the gastro-intestinal tract of fish resulted in a 2-fold reduction of the risk (WI-6).

We did not consider the risk of a new TSE infection in fish due to exposure to BSE infectivity in aquafeed. This risk is considered very low given that (1) based on low sequence homology of prion proteins (PrP^C) in fish and mammals, the species barrier between bovines and fish is deemed to be high (Matthews and Cooke, 2003; Friedland et al., 2009; Pers. comm. L. Van Keulen), (2) experimental studies in fish did not result in TSE infections despite exposure to high infectivity loads (SSC, 2003; Ingrosso et al., 2006; Salta et al., 2009), and (3) routine examination of fish brain in the course of fish disease diagnosis never raised suspicion of TSE in fish (SSC, 2003). Given the estimated low exposure of fish to BSE infectivity in aquafeed in this study (median of 0.24 $CoID_{50}$; 95 % uncertainty interval 2.5×10^{-3} – 3.0), and the expected high species barrier, it is unlikely that inclusion of bovine SDRBC in aquafeed would result in BSE infection in fish indeed. Furthermore, to the best of our knowledge inclusion of bovine SDRBC in aquafeed in countries outside the EU never resulted in detection of TSE infection in fish. However, if a TSE infection in fish would arise, and transmission would resemble BSE transmission in cattle, this could be of great concern given the high ration of fishmeal in aquafeed (both intra-species and intra-order recycling of fishmeal). The resulting infection risk for calves from inclusion of fishmeal in calf milk replacer is, however, difficult to estimate, since there will then be a reversed species barrier from fish to cattle.

In the model, a homogenous distribution of BSE infectivity in both the contaminated tissues of an infected animal, and the different products produced along the pathway from slaughtered cow to calf milk replacer was assumed. If, however, clumping of prions would occur, the infectivity in the different steps of the model could be either lower or higher. Clumping of prions could result in a higher infectivity load at the end of the pathway if, e.g., infectivity in brain tissue was clustered or if infectivity is not equally distributed across the different outcomes of each step in the pathway. Although what-if scenario WI-14 accounted for a single destination of a contaminated batch of bovine SDRBC (all processed in aquafeed) and a contaminated batch of fishmeal (all processed in calf milk replacer) rather than clumping of prions, its results also indicate the effect of clustering of infectivity resulting in accumulation in a single path of the pathway. Clumping of infectivity could also result in a higher probability of infection of individual animals, if it results in exposure to a higher infectivity load. This is especially important if a minimum infectious dose is required to result in infection, something which is currently not known (EFSA, 2020). The exponential dose-response model (Eq. (8)) that we used to estimate the probability of infection is a worst-case approach, as it assumes that there is no threshold dose for prions (Gale, 1998). The final dose of BSE infectivity to which individual calves will be exposed due to consumption of contaminated calf milk replacer will probably be at least 2 $\log_{10}$ lower than the estimated BSE infectivity in calf milk replacer (I_{CMR}), as the contaminated calf milk replacer will be distributed among multiple calves. If the resulting individual dose is below the minimum infectious dose, exposure will not result in infection at all.

The model calculations did not differentiate between classical BSE (C-BSE) and atypical BSE strains (H-BSE and L-BSE), as we did not have quantitative data to make a distinction in the risk of these different BSE strains. Most input parameters were based on data for C-BSE. Feeding of infected material is considered an important route of transmission for C-BSE, which was evidenced by the rapid decline of BSE infections after implementation of the total feed ban in 2001 (EFSA, 2018; Kumagai et al., 2019; Alarcon et al., 2023). The transmission routes of H-BSE and L-BSE have not been elucidated yet, if these strains are being transmitted at all (Dudas and Czub, 2017). So far, epidemiological observations do not support transmission of the atypical BSE strains and the hypothesis is that infections occur spontaneously (CFSPH, 2016; Kumagai et al., 2019; WOAH, 2023a). Transmission of these strains by feed can, however, not be completely ruled out when TSE regulations, such as the feed ban, species-to-species ban and removal of specified risk material, would be lifted. Although the oral dose needed for successful transmission of atypical BSE is higher compared to classical BSE (Okada et al., 2017; Kumagai et al., 2019), atypical BSE has been hypothesized as a possible origin of classical BSE (Dudas et al., 2023). Therefore, we argue that based on the current knowledge and from a risk-averse perspective, the BSE risk of including bovine SDRBC in aquafeed should be considered equal for C-BSE, H-BSE and L-BSE.

Conclusions

This study illustrates how a quantitative risk model can be used to evaluate the BSE risk of using residual streams derived from bovine sources as an ingredient in animal feed, which is a prerequisite to assess the safety of introducing such residual streams in a circular food system. Model results indicate that the expected BSE infectivity in blood from a BSE-infected cow at the clinical end state of infection is <1 $CoID_{50}$. Infectivity in blood mainly results from cross-contamination with brain tissue during stunning at the slaughterhouse. The initial infectivity is reduced along the pathway from slaughtered cow to calf milk replacer.

The highest reduction is achieved by clearance of infectivity by fish fed bovine SDRBC as an ingredient of aquafeed, although this parameter has high uncertainty. The final infectivity reaching calves via inclusion of fishmeal in calf milk replacer is estimated to be very low (median value: 1.1×10^{-5} $CoID_{50}$). Assuming an exponential dose-response model, this corresponds with an expected probability that < 10 out of a million BSE-infected cows slaughtered at clinical end state will result in new BSE infections, which is far below the threshold value of 1 for the basic reproduction number (R0) to initiate a new epidemic. We thus conclude that it is very unlikely that allowing the use of bovine SDRBC as ingredient of aquafeed will result in a new BSE epidemic in cattle. What-if analysis indicated that this conclusion is robust, despite high uncertainty for some input parameters.

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CRediT authorship contribution statement

C.J. de Vos: Writing – review & editing, Writing – original draft, Validation, Software, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **A.F.G. Antonis:** Writing – review & editing, Validation, Investigation, Conceptualization. **M.H.J. Sturme:** Writing – review & editing, Project administration. **M. Appel:** Writing – review & editing, Project administration, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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