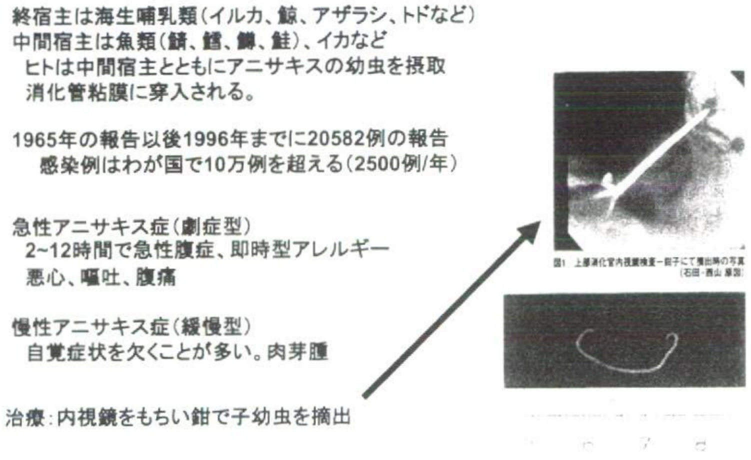

図1　アニサキス症の幼虫の移行症

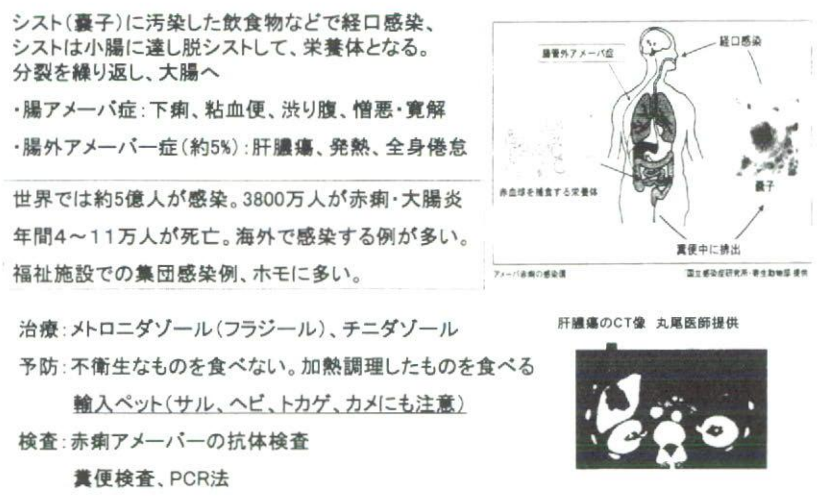

図2　アメーバー赤痢

いるサケ，マスから感染，海生哺乳類が宿主と考えられる)

・マンソン裂頭条虫症（アジアに分布，第一中間宿主はケンミジンコ，第二中間宿主は両生類，爬虫類，鳥類，哺乳類，ヒトはプレロセルコイドが感染しているヘビ，ニワトリの生食，成虫はイヌ，ネコに寄生)

・有鉤条虫症，無鉤条虫症，アジア条虫症（ヒトが終末宿主，有鉤条虫・アジア条虫はブタ，無鉤条虫はウシが中間宿主，食肉・内臓により感染)

原虫症：

・アメーバ赤痢（ヒトーヒト，サル類ーヒト)：サル類，イヌ，猫，齧歯類も保有

・クリプトスポリジウム症（ヒト型はヒト，霊長類に感染，他にウシ型，イヌ型もヒトに感染する。オーシストに汚染した飲食物を介して感染，10人／年)

・ジアルジア症（ランブル鞭毛虫症，世界では50

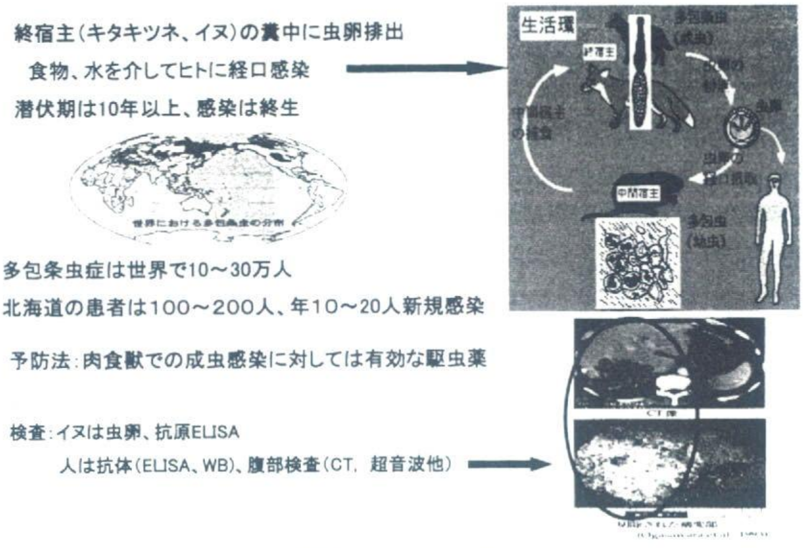

図3　エキノコックス症

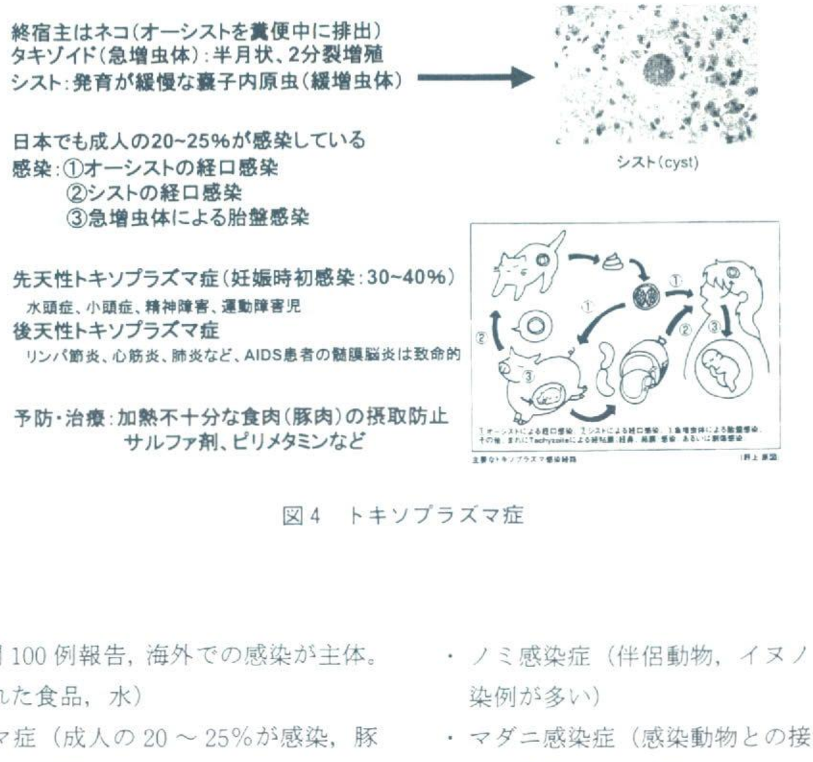

図4　トキソプラズマ症

万人／年，年間100例報告，海外での感染が主体。嚢子に汚染された食品，水）

・トキソプラズマ症（成人の20～25%が感染，豚肉から感染，ネコが宿主）
・バベシア症（ウシ：*B. bovis*，野生齧歯類：*B. microti*，バベシア原虫に感染したダニの刺咬，日本では1例報告あり齧歯類由来）

外部寄生虫症：

・疥癬（穿孔ヒゼンダニ感染症，イヌ，ネコ由来）
・ノミ感染症（伴侶動物，イヌノミ，ネコノミの感染例が多い）
・マダニ感染症（感染動物との接触，マダニ生息地への侵入により感染）

身近にある動物由来感染症と寄生虫感染症

身近にある動物由来感染症でもっとも危惧されるものは，やはり狂犬病である。発症したら100%死亡する。幸いかまれた後でもワクチンが有効（暴露

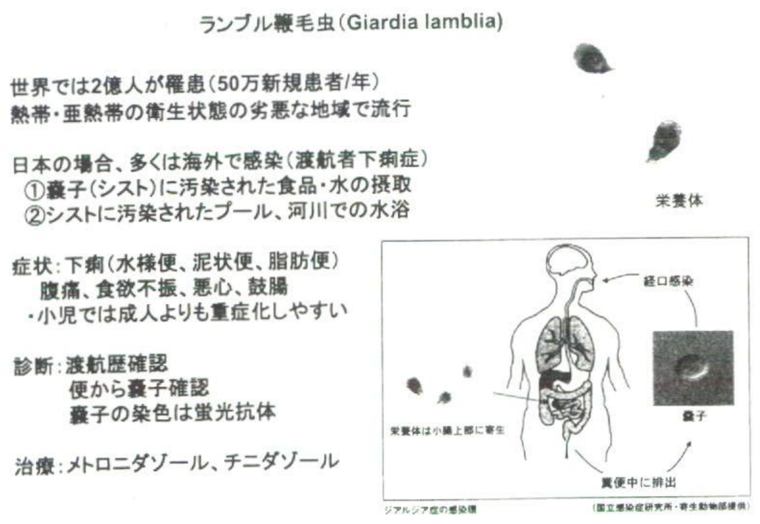

図5　ジアルジア症

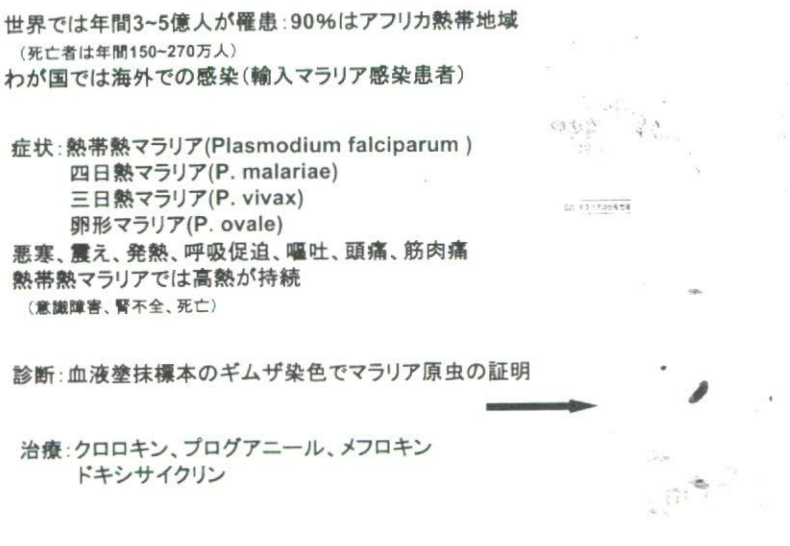

図6　マラリア

後ワクチン）であり，発症を阻止できれば，治癒可能である。国内では約半世紀，流行は見られないが，2006年，日本人2名がフィリピンで感染し，帰国後に発症し死亡して話題となった。

死亡することもある感染症で動物－ヒト－ヒト（まれに人から人に感染する）感染を起こすものにはオウム病があり，近年では松江のフォーゲルパークで流行，神奈川の動物園のヘラジカの出産に立ち会った獣医師などの感染が報告されている。動物－ヒトで重症化するものにはエキノコックス症がある。また場合により重症となるものでは動物－ヒト感染ではトキソプラズマ症，アメーバ赤痢（人から人への感染が報告されている），トキソカラ症，ライム病，レプトスピラ症がある。他に，日和見感染が基本であり，免疫機能低下の場合に問題となるものにクリプトコッカス症，Q熱，パスツレラ症，ネ

コひっかき病，サルモネラ症などがある。ここで，寄生虫感染症に分類されるものは，エキノコックス症，トキソプラズマ症，アメーバ赤痢，トキソカラ症である。

また医師が問題とするペット由来の感染症（医師191例回答）では，皮膚白癬症（内科21例），イヌ回虫症（内科2例，外科3例），トキソプラズマ症（内科3例，外科12例），キャンピロバクター症（内科2例），ネコひっかき病（内科12例，外科21例），ダニ症（内科1例），オウム病（内科21例，外科2例），サルモネラ症（内科8例）が上げられており，寄生虫感染症としては，トキソカラ症，トキソプラズマ症，ダニ症がリストアップされる。

他方，感染症法の届出対象では広義の動物由来感染症として，赤痢，腸管出血性大腸菌症（O157），アメーバ赤痢，エキノコックス症（単包条虫を含む），ジアルジア症，オウム病，Q熱，ツツガムシ病，日本紅斑熱，ライム病，日本脳炎，デング熱，マラリア，クリプトスポリジウム症，ブルセラ症，レプトスピラ症，E型肝炎などのヒトの症例が報告されている。このうち4種類の届出対象となる寄生虫感染症は新規患者の発生数として，アメーバ赤痢が毎年300～600人，エキノコックス症が10～25人，ジアルジア症が40～140人，マラリアが70～150人である。

ここでは，感染症法にとらわれず，身近にあって知っておきたい寄生虫感染症としてアニサキス症，アメーバ赤痢，エキノコックス症，トキソプラズマ症，ジアルジア症，マラリアを紹介した。

寄生虫感染症のリスクとその対応

数年前に輸入動物に由来する動物由来感染症のリスク評価を，厚生省のワーキンググループで初めて試みた。その結果として，輸入禁止動物，法定検疫の必要な動物，輸入届出の必要な動物等が決められ，法制化された。実施後1年の有効性評価では，輸入動物数が減少したこと，野生動物の輸入がほぼ完全に止まったこと，中近東やアフリカからの動物輸入がなくなったことなど，リスク回避措置が有効であったことが明らかにされた。

しかし，こうしたリスク評価を寄生虫感染症について行う前に，寄生虫と人の関係を生物学的に考えると，10～20億年の歴史を持つ原生動物，あるいは扁形動物，線形動物とわずか500万年の歴史しか持たない人（チンパンジーから分岐して500万年，最後にアフリカ大陸から発した現世新人類はわずか6万年の歴史を持つに過ぎない）の関係である。火を使い環境を改造してきた人類が，今後も寄生虫に戦いを挑むとすれば，このような進化の歴史を無視して挑戦することは無謀であるし，清浄化しすぎる危険性もまた考慮する必要がある。この点で，2つのことを指摘しておきたい。1つは生態的ニッチの問題である。あまりにきれいになりすぎて，生態学的空白地域ができると，そこを埋める生物が出現する。その場合，敵対的生物による抑制がないと爆発的な増殖を可能にする。日本のように極端に清浄化された地域では，新しい寄生虫が侵入した場合，大流行を起こす危険性がある。第2はリスクシナリオである。地球温暖化の影響による寄生虫媒介ベクターの北上，また，野生鳥類・哺乳類の輸入規制が両生類・爬虫類輸入の増加へシフトした場合，これまでにない寄生虫の中間宿主の輸入増加に繋がる可能性がある。さらに慢性的な食料自給率低下は，寄生虫を含む汚染食品の輸入量増加へ繋がる。このようなことは，今後の対策として考えておかなければならない。

おわりに

現在のわが国の寄生虫感染症で問題となるものの多くは，国外での感染である。極端に清浄化された環境である日本の常識は世界の非常識？と考えて，海外旅行を楽しむ必要があろう。また，公衆衛生（集団防衛）行政と個人（患者）とでは，リスクの考え方が違うことは明らかで，公衆衛生上，重要でなくても罹患したヒトにとっては100%のリスクである。その意味でも「たかが寄生虫されど寄生虫」である。しかし，多くの寄生虫感染症は適切な予防と治療で制御できるし，そのためには正確な情報と正しい知識（教育・啓蒙）が必要となる。

畜産システム研究会報　第31号：1－14（2007.12）

BSE の感染源および感染経路に関する疫学研究報告書の概要

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Epidemiological study on BSE in Japan
— Reconsidering of BSE in Japan —
Symposium by Society for the Study of Animal Husbandry

Graduate school of Agricultural and Life Sciences, University of Tokyo
YOSHIKAWA, Yasuhiro

1. Expansion and transmission of BSE and vCJD

Time sequentially, the BSE outbreaks are separated into three categories in the world. They are in the UK, EU and other countries. BSE might be emerged in early 1980s in the UK, and first identified on 1986 and then confirmed in the general meeting of the OIE (Office de International Epizootics; World Animal Health Organization) on 1988. Meat and bone meal (MBM) including greaves was considered as a causative material by epidemiological studies done in the UK[1] and the materials were banned to be used for animal feed as early as 1988. The specified risk material (SRM: SBO; specific bovine offal) was removed from the food chain in the end of 1989. During this time, live cattle and the extra MBM were exported from the UK to the EU, mostly. The feed ban was relatively effective, and BSE positive cases were peaked in 1992 and 1993, and gradually decreased later. However, in 1996, new variant Creutzfeldt Jacob Diseases (vCJD) were reported and it induced a world threatening panic. The UK government decided to start a measure of incineration of over thirty month cattle (OTM), and both exportation ban and real feed ban of MBM. From 1996 to 2000, 4.5 million cattle were burned and the UK succeeded to contain the BSE. In 2005, OTM regulation was relaxed to the BSE screening test in the slaughterhouse.

The EU countries recognized the risk of BSE and stopped importation of MBM from the UK on 1990, and as a result extra MBM of the UK was exported to Asia, America, and East European countries. The EU started a feed ban on 1994 and extra MBM was exported to other countries too. BSE contamination in the EU might be peaked during 1995 and 1996. In 2000, the EU started the active surveillance (rapid BSE test of cattle in the fallen stock and slaughterhouse), and introduced the real feed ban on 2001. In 2002, BSE positive cases detected by the active surveillance became a peak, and rapidly decreased later. In 2006, the EU accepted the deregulation of OTM of the UK, and started a re-importation of UK beef.

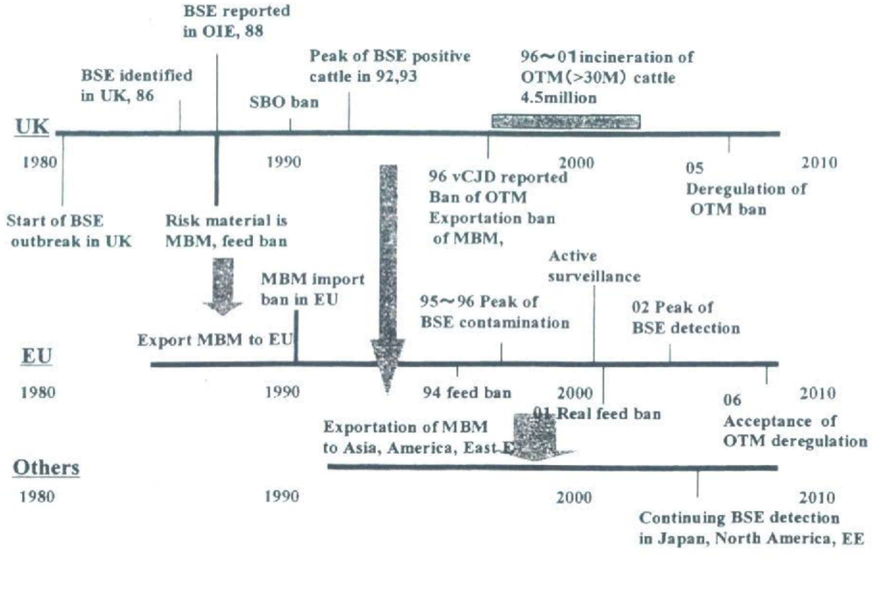
The third group, such as Japan, North America and East European countries etc., which imported live cattle, MBM or greaves from the UK and EU, were involved in BSE outbreak later than 2000 and detection of BSE cattle are still continuing now.

BSE positive cases reported in the World at 2007 January were as follows. The UK had more than 180,000 cases and the number is extremely high, and three EU countries, Ireland, Portugal, and France, detected 1,000 to 1,500 BSE cattle. Spain, Germany and Switzerland reported about 500 cases, and Italy, Belgium and Holland had about 100 cases. Japan reported 31 BSE cases, but now in October 2007, the number is 33. The total number of countries with BSE positive cases in the world is now 25.

Case reports of vCJD were a bit different from those of BSE. The UK had 164 patients at 2007 January, and they were relatively young (20 to 30 years old). In France, the number of BSE was not so high (about 1,000) when compared with the UK (180,000) and Ireland (1,600) or Portugal (1,000), but number of the vCJD patient was high, 21 cases, when compared with Ireland (4) and Portugal (1). Moreover, the age of patients is older (30 to 40 years old) than that of the vCJD in the UK. There are several possibilities on a high frequency of vCJD in France. That is a large quantity of contaminated meat product importation such as machinery recovery meat (MRM) from the UK, or dietary culture of the calf brain consumption. The total number of countries with vCJD patients in the world is now 11. From 2003, the vCJD entered into a new phase in the UK i.e., transmission of vCJD by blood transfusion was reported, and 4 cases were detected in the UK until now.

Thus, from 1986, BSE transmitted from cattle to cattle by MBM in the feed, and one full brown BSE cattle was considered to have an infectivity of 4,160 CoID50 from the calf infection experiments by the EFSA (European Food Safety Agency)[2]. And complete feed ban, that is incineration of SRM, is the most effective measure. On the contrary, incomplete feed ban such as stop of ruminant MBM to supply to ruminants, results in cross contamination (cc). For example if one full blown BSE cattle, which has 4,160 $CoID_{50}$ infectivity was rendered by the ordinary methods, the infectivity titer reduced to x 0.1 (by rendering), and probable quantity of the cross contamination is x 0.005 = 2.1 BSE cattle. It means one infected full blown BSE cattle might produce 2 positive cases by rendering within a

Back ground of BSE outbreak in the world



mean incubation period.

From 1996, vCJD cases transmitted by consumption of the SRM of BSE cattle were reported. The first epidemics, in which the people with prion gene of 129 M/M homozygote were involved, was a peak in 2000 to 2001 and then the number reduced. The SRM elimination from food chain is a principle countermeasure, and an elimination of BSE cattle which were born before real feed ban by the BSE test in slaughter house is needed.

From 2003, transmission of vCJD by blood transfusion was reported of 4cases in the UK. In the case of BSE, the prion is restricted to the central nervous system or neuronal tissues. But, in the case of vCJD, the prion exist not only nervous tissues but also lymphoid tissues and blood circulation. By the Fisher's Exact Test comparing rate of infection after transfusion from vCJD (4case vs. 14) and CJD donors (zero vs. 116) suggests a statistically significant difference between two groups (< 1% likelihood). It suggested that vCJD is more easily transmitted than ordinal CJD, which had been known to cause the iatrogenic infection.

2. List of BSE positive cases in Japan

The 33 BSE positive cases reported in Japan until 2007 October were divided into 6 groups by a temporal and spatial difference. Pre-A-group is one case of Japanese black, female beef cattle which was born in the Iki, a small island of Nagasaki prefecture (Kyushu) in 1992 Feb. She was atypical BSE and detected 169 months old of age. This case might be independent from the outbreak of other typical BSE groups in Japan. Recently, the prion of this case was confirmed to be transmissible to transgenic mice with bovine prion gene (Tg bov)[3].

The A-group consisted of 13 typical BSE cases, and the characteristics of this group might be involved in a concentrated BSE contamination for a short term and all the dairy cow (Holstein) were born before or just after the administrative guidance for feed ban in 1996 April. The 10 cases in Hokkaido were born from 1996 February to August, and 3 cases in Kanto were born from 1995 December to 1996 March.

The B-group is one case of Holstein dairy cow which was born 1999 July in Kumamoto prefecture (Kyushu). She was the typical BSE case

Transmissibility of BSE and vCJD

From 1986
・BSE transmitted from cattle to cattle by MBM (greaves)
/ One full blown BSE cattle has an infectivity of 4160 $CoID_{50}$
/ Complete feed ban (incineration of SRM) is the most effective measure
/ Incomplete feed ban results in cross contamination (cc)
1 BSE cattle, 4160 (ID_{50}) x 0.1 (rendering) x 0.005 (cc)=2.1 BSE cattle

From 1996
・vCJD transmitted by consumption of the SRM of BSE cattle
/ SRM elimination from food chain is principle
/ Elimination of BSE cattle by BSE test in slaughterhouse

From 2003
・Transmission of vCJD by blood transfusion (4cases in UK)

	Infection	No infection
vCJD	4	14
CJD	0	>116

Fisher's Exact Test comparing rate of infection after transfusion from vCJD and CJD donors suggests a statistically significant difference between two groups (<1% likelihood): by Dr. Asher of FDA

and no direct relation was considered with Pre-A-group cattle born in Nagasaki prefecture (Kyushu).

The C-group is now going to be a peak of BSE detection age. Until now, 16 cases were reported. The characteristics of this group are the all cattle were typical BSE and born exclusively in Hokkaido during 1999 August to 2001 August, before when the real feed ban was started (2001 October). The 15 cases were Holstein dairy cow and one case was female beef cattle of the Japanese black which was reared with dairy cow in the same farm by a compound management. Thus, she consumed the same milkreplacer, calf starter and the mixed feed as the dairy cow.

The D-group consisted of 2 male young cattle of Holstein and both animals were born just after the real feed ban on 2001 October. One was born at 2001 October in Tochigi prefecture (Kanto) and became BSE test positive on 23 months old of age. He was diagnosed as an atypical BSE case. The other animal was born at 2002 January in Hyogo prefecture (Kansai) and became positive on 21 months old of age. He was belonged to the typical BSE case. In both cases, accumulation of the prion in the obex was very little and the prion were tried to transmit to the Tg-bov for two generations by blind passages but the results were negative[4].

The Post-D group including cattle born after 2002 February, there were no BSE positive cases until 2007 October.

3. BSE safe guard policy and countermeasures of BSE in Japan

After the first case of BSE was detected in Chiba prefecture (Kanto), consumers were led into a very big panic. The reason is that they lost trusts to the government and others as follows. There was distrust to the government because MAFF (Ministry of Agriculture Forest and Fishery) made a misunderstanding of the risk of BSE invasion, a lacking crisis measures, and no risk communication. Consumer gave a strong claim to the cattle breeder too, because they used MBM as cannibalism for cattle, and gave a priority to the economy rather than safety. At the same time, meat importer and processor conducted false applications for the compensation buying of the domestic beef by the MAFF, and false tags of meat by the distributor in the beef market were also disclosed. Every day TV and

Grouping of BSE positive cattle

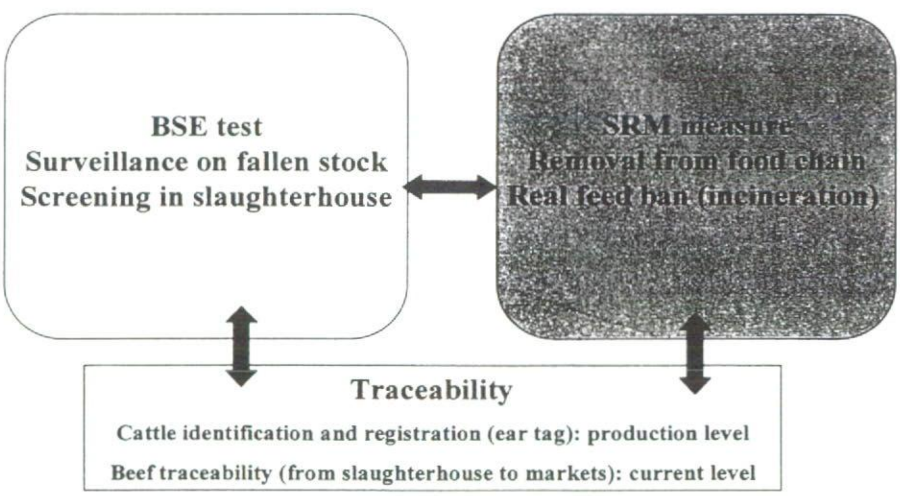
Group (n)	Birth date	Birth place	Race	Characteristics
Pre-A (1)	1992 Feb	Nagasaki Iki island	Japanese black ♀	·**Aged (169 M), Atypical case** ·No relation with A group
A (13)	1995 Dec~ 1996 Aug	Hokkaido Kanto	Holstein ♀	·1996 Feb to Aug in Hokkaido (10cases) ·1995 Dec to March in Kanto (3cases) ·All BSE cattle were born before or just after the administrative guidance for feed ban in 1996 April
B (1)	1999 July	Kumamoto	Holstein ♀	·Only one case ·No relation with the Nagasaki pre-A group ·No direct relation with Hokkaido C group?
C (16)	1999 Aug~ 2001 Aug	Hokkaido	Holstein ♀ Japanese black ♀	·Holstein 15 cases ·Japanese black 1 case (Dairy cow and beef cattle were reared in the same farm) ·All cattle were born before real feed ban on 2001 October
D (2)	2001 Oct 2002 Jan	Tochigi Hyogo	Holstein ♂ Holstein ♂	·**Juvenile (23 M), Atypical case** ·Juvenile (21 M) ·They were born just after real feed ban on 2001 October
Post-D (0)	After 2002 Feb			

newspapers mediated noisy but less signal information (low SN ratio). And finally, consumers distrusted the scientist who denied ZERO-risk and showed a scientific uncertainty, because majority of people believed that science is almighty, can explain everything and ensure safety. Thus, the consumers had an anxiety when they faced a scientific uncertainty.

Japanese government's policy of safe guard for BSE was consisted of three elements. The first is the SRM measures. The SRM of all aged cattle including the brain, trigeminus ganglia, spinal cord, vertebral column containing dorsal root ganglia, distal ileum, and tonsil/lingual tonsil are removed from food chain and incinerated. Besides SRM, other parts which are not used for human-consumption are rendered into MBM and incinerated, too. Thus, complete feed ban was performed. The second is BSE test which purposed surveillance of BSE on all fallen stock > 24 month old and screening of BSE cattle in the slaughterhouse > 21 month old. The third is traceability system. All cattle are identified by ear tag number with 10 digits and registered in the national control center in production level. A small portion of the all cattle meat from carcasses was stocked for the DNA diagnosis and 10 digits were displayed in the market or meat house. This is the beef traceability from the slaughterhouse to the markets in current level. The 10 digit can be traced by a personal phone or internet.

Another government policy introduced after BSE outbreak in Japan is separation of risk assessment and management; that is an introduction of risk analysis system on food safety. Risk analysis consists of three elements, i.e., risk assessment, risk management and risk communication. In Japan, risk assessment is done neutrally and scientifically in Food Safety Commission (FSC) of the Cabinet Office. The purpose is scientific assessment of hazards for human health. On the other hand, risk management is done politically by MAFF or MHLW (Ministry of Health Labor and Welfare). The purpose is making a standard or regulation based on the risk assessment with considering risk benefit or cost benefit. Risk communication is an exchange of

Government Policy on Safe guard for BSE in Japan



information or opinion about the risk by all stake holders and it is performed by public comments and risk communication meetings.

Although there occurred a nationwide panic among consumers after the first BSE case, the activities of three organizations (MAFF, MHLW, FSC) has been successful in obtaining confidence or trust of consumers relatively short time. In 1996 MAFF started BSE surveillance test of the risk animals, and the TSE including BSE was involved in the notification disease in 1997. After the first case of BSE, complete feed ban for the use of MBM, removal of SRM and BSE test in the slaughterhouse had been obligated (2001 October). In addition, BSE-related risk is assessed by the Prion Expert Committee, Food Safety Commission, in the Cabinet Office since July 2003. Then, introduction of traceability system, and several BSE related risk assessments were conducted[5].

Basic strategy of the risk management for BSE in Japan is importation ban of MBM, live cattle and beef from the countries where BSE has been detected. Cattle are grown with the feed produced in the completely isolated facility from those producing pig or poultry feed in order to avoid cross contamination. In the farm, all cattle are identified by the traceability system and fallen stock in the farm over than 24 month old is BSE tested. In the slaughter house BSE test is conducted to over than 21 month old cattle by the law and the positive cattle is incinerated. The SRM is removed from all cattle regardless of the result of BSE test and incinerated. Both the slaughterhouse and meat processing facilities are completely separated between cattle and pig meat to avoid cross contamination. Non human-consumption materials from cattle are rendered and finally incinerated.

Thus, containment of the BSE risk is based on stopping cattle to cattle, cattle to human and human to human infections. These are MBM feed ban, control of feed production and compost regulation. BSE test and SRM removal in the slaughterhouse, and regulation for drug production using BSE free cattle materials are safeguards for cattle to human infection. Ban of blood transfusion and organ transplantation

BSE countermeasure in Japan

Cattle-cattle

MBM
Feed ban and incineration of cattle MBM by the law (01)
Use of swine MBM for swine feed after separation of feed factory (05), for fish feed (07)

Feed production
All feed factories have exclusive production facility or line (04)
Enforcement of notification of imported compound feed (05)

Compost
Liquid compost (high pH) from cattle MBM (04)
Ash of cattle MBM or bone can be used for compost (05)

Cattle-human

Meat/visceral organs
BSE test on all ages of cattle in slaughterhouses (01)
SRM removal (01)
Vertebrate becomes SRM (04)
BSE test in slaughterhouse became >21M by law (05)

Drugs
Bovine material importation ban from UK (96)
Importation ban from high risk area (BSE positive area:00)
Use only low risk country derived category III, IV tissues (01)

Human-human

Transfusion,organ transplant
Transfusion ban from the person stayed in UK during 80～96 for >6M (99)
Ban of transfusion & organ transplant from the person after 1980 in UK, France, Germany, Swiss,Ireland, Portugal, Spain for >6M (01)
Expand to all European BSE positive countries (03)
Expand to person stayed >1d in UK until 96 (05)

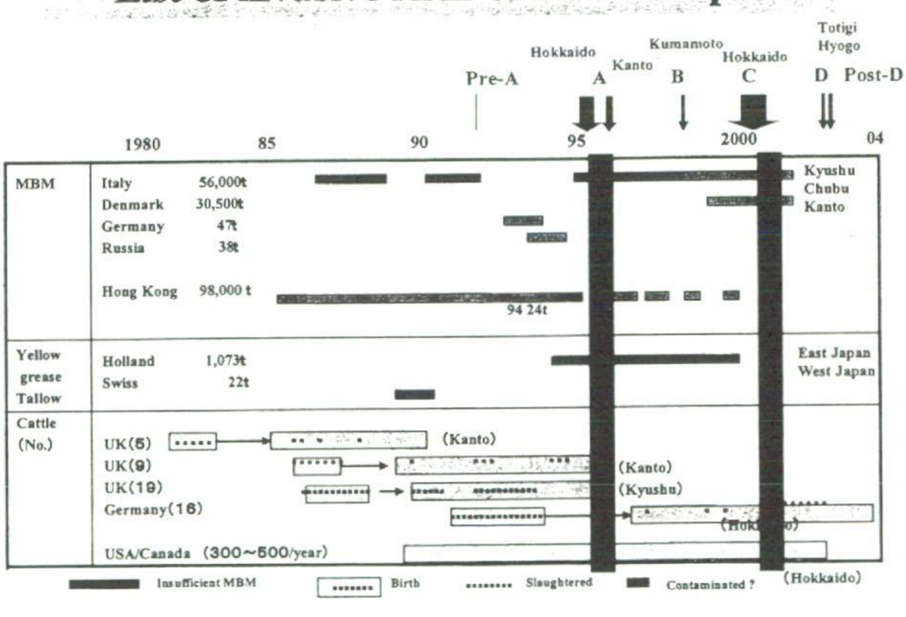
from the high risk person who stayed in the UK until 1966 or EU, is precautious to prevent human to human infection. Japan is one of the most strictly regulated countries in the measures of BSE, but we have already conducted deregulations of some measures, too. They are use of swine MBM for swine feed after complete separation of feed factory or feed producing facilities (2005) and use for fish feed (2007), after risk assessment of FSC. Liquid compost treated with high pH from cattle MBM (2004), and ash of cattle MBM or bone can be used for the compost (2005). And revision of BSE test in slaughterhouse with more than 21 month old was settled in law (2005) by the assessment of FSC.

4. Invasive BSE risk scenarios in Japan

The main work of this study is to identify, as hypotheses, all feasible sources and routes of infection for the BSE cases discovered in Japan, and to study the probability of each hypothesis. Unlike ordinary microbial infections, BSE has an extremely long incubation period for several years (mean incubation period is Ca 5 ± 1.5 years). It is technically impossible to detect genome of pathogen or antibody, and there are extremely few confirmed cases. Nevertheless these difficulties, it is hoped that these research results will be of use in preventing future outbreaks, which is the primary object of the epidemiology.

The principle and strategy of this epidemiological study of BSE in Japan are as follows. 1) BSE risk status in Japan is divided into 3 sages, i.e., before 1996 April when administrative guidance for feed ban of cattle MBM introduced. After that to 2001 September, the first case of BSE in Japan, then, after 2001 October with real feed ban in the law. 2) Make hypotheses depending on the invasive risk and propagation risk of BSE in Japan, and they are checked by evidences, case control study or statistics. 3) Grouping of BSE cattle was conducted time sequentially and spatially; that is Group-A (1995, 1996, born in Hokkaido, Kanto), Group-B (1999 born in Kyushu), Group-C (1999-2001 born in Hokkaido),

List of invasive risks of BSE in Japan



Group-D (young cattle born after real feed ban) and Pre A- , Post-D groups.

The risk of the BSE agent being introduced to Japan through imports of the live cattle can be divided into four scenarios. These are the 5 cattle born in southern England and imported into the Kanto region in 1982, the 9 cattle born in southern England and imported into the Kanto in 1987, the 19 cattle born in southern England and imported to Kyushu in 1988, and the 16 cattle born in Germany and imported to Hokkaido in 1993. All history of each animal in Japan is known. On studying the respective import lots, the origin of live cattle imports from the UK (all dairy cattle) gradually shifted from central to southern England, where BSE contamination was more intense. This study also clarifies issues such as where the cattle were reared in Japan after import, i.e. the slaughterhouse processing after exhaustion of the reproductive cycle, MBM manufacturing processes, and where the cattle were re-used as MBM. In Japan, over 160 slaughterhouse and rendering factories, as well as feed factories are regional industries. Almost all animal feed were produced and consumed in local regions, for example Hokkaido feed products are 100% consumed in Hokkaido.

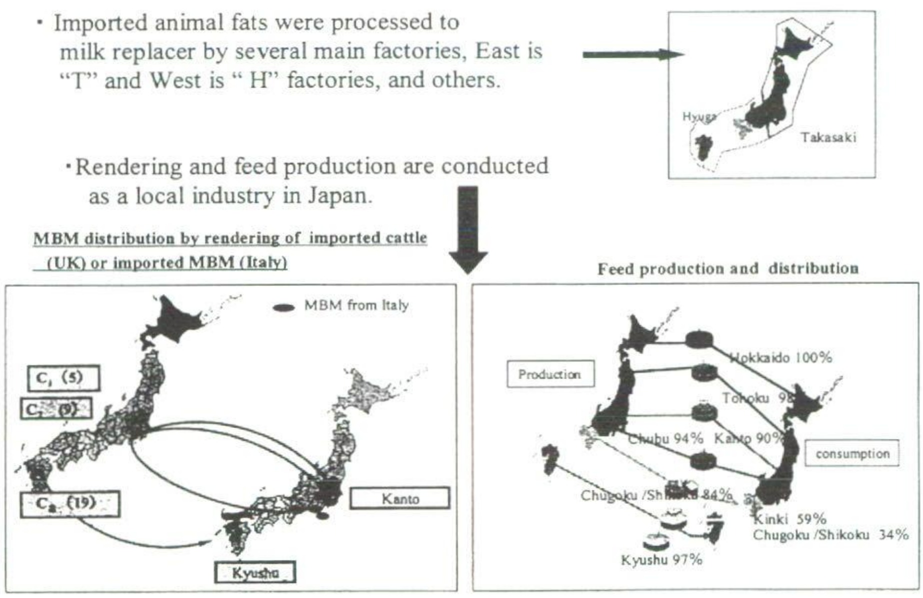
The risk of imported MBM is divided into three scenarios, i.e. imports from Italy, Hong Kong and Denmark. The scenarios for MBM imports from Germany or Russia were not considered, since the risk from these is thought to be extremely low. This is because the respective import volumes were small and there was hardly any manifestation of BSE among cattle produced in those countries at the time of import. In the case of Italy, 55,930 tons of Italian MBM had been imported between 1987 and 2001. Moreover, MBM imports from Italy involve a number of factors, including the state of BSE incidence in Italy, variations in the volume of MBM imported, and changes in the MBM manufacturing processes. Namely, the 656 tons imported between 1987 and 1993 (no imports in 1991 and 1992) to Yokohama (Kanto), Nagoya (Chubu) and Moji (Kyushu) were categorized as M1, the 5,408 tons imported between 1995 and 1998 to Nagoya (Chubu), Yokohama (Kanto) , Kobe (Kansai) and Kagoshima (Kyushu), of which the 4,802 tons imported after June 1998 were heat-treated at 133℃, 3 bar, 20 minutes as M2, and the 49,846 tons imported between 1999 and 2001 to West Japan (Kyushu, Kansai, Chubu) as M3. In the case of Denmark, 30,500 tons of Danish MBM had been imported in 1999-2000.

The risk of importation of animal fat and tallow is divided into two scenarios. These are the 1,245 tons of Holland animal fat (powdered fat) imported into Kanto and Kyushu between 1994 and 2000, and the 22 tons of Switzerland animal fat (tallow) imported in 1989. Imported animal fats were used for producing milkreplacer. In Japan, there are a few milkreplacer factories and they shared broad area such as East of Japan or West of Japan.

The BSE risk status of Japan was classified tentatively into three periods as follows by the BSE risk assessment. Before 1996 April, there ware no regulations for rendering and animal feed, and the invasive risk was high because importation of live cattle from the UK was moderate, Italian MBM was high, and animal fat from Holland was very low. The stability was extremely unstable, because the rendering condition was poor (110℃, 1 bar, 60 min) and SRM was used for the cattle feed. Thus BSE risk status of this period is GBRIII or undetermined. The period from 1996 April to 2001 September, invasive risk was moderate because live cattle importation from USA and Canada is low, MBM imports from Italy and Denmark was moderate and animal fat from Holland was very low. The stability was unstable because partial feed ban was introduced (1996 April, Administrative guidance for feed ban of cattle MBM) but SRM was used for rendering and feed production was conducted with a condition of cross contamination. Moreover, MBM was used for a supplement in many dairy farms. Thus, BSE risk status of this period was GBRIII or undetermined. After 2001 October, the BSE risk status became low ~negligible, because invasive risk of live cattle from USA and Canada was low, complete ban of MBM importation was negligible risk and animal fat importation from Australia was negligible risk. The stability was very stable because SRM was incinerated. Moreover, slaughterhouse, rendering facility and feed factory were separated between cattle and pig or chicken systems avoiding cross contamination. Thus, BSE risk status of the period is GBRI or the controlled risk status.

5. Characteristics of BSE outbreak in Japan

The feature of the BSE outbreak in Japan is a bit different from those of European countries as follows. 1), Epidemic size is relatively smaller than those in European countries. Extremely intensive BSE test had been conducted in Japan and a nationwide traceability system by the MAFF was established. Thus, precise data on each BSE positive case could be listed up, and all population of cattle including healthy slaughterhouse animals (from 2001 October) and farm fallen stocks (from April 2004) were BSE tested. 2), Un-even distribution of BSE cases was observed. Until now, Hokkaido is a core of BSE epidemic in Japan, and two different time outbreaks were occurred there. 3), Dairy cow are mainly involved. And 4), Sporadic, discontinuous outbreaks were occurred temporally-spatially. That is, in 33 BSE test positive cattle in Japan, the birthplaces of 26 cases are Hokkaido and other 7 animals are born in other prefectures. If atypical cases were eliminated (case No. 8 and 24) from the outbreak of typical BSE, 26 in 31 cases (84%) were born in



Hokkaido.

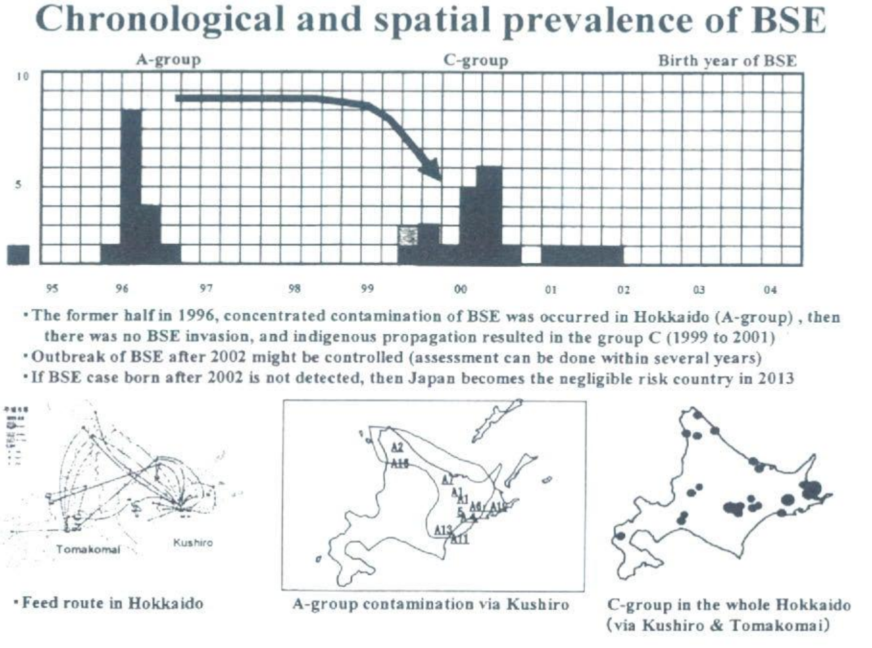
Another characteristic of relatively big BSE outbreaks in Hokkaido is difference of the temporal patterns of the birth dates in cattle of the Group-A and -C. Mean and standard deviation of the A-group birth interval are 3.6 ± 1.8 M. It is concentrated in an extremely narrow period. However, those of the C-group are 10.5 ± 6.6 M until now. By the statistical analysis, the group-A & -C are parametric, but uneven distribution, in the 95% interval, P (both sides examination) = 0.0013. It suggested that the cause of A-group might be different from that of C-group significantly, and possible contamination of A-group might be single or a few number of lots of the causative agents. Special distribution of BSE cases in A-group Hokkaido was closely related to the milkreplacer and feed distribution route by the Kushiro-route.

Population study on the Hokkaido dairy cows suggested followings. About 160,000 to 170,000 dairy cows are born every year in Hokkaido and majority of the population are slaughtered from 4 to 7 years old of age (total ~65,000 head per year). Mortality rate of the fallen stock is high during 2 to 7 years old of age (total ~30,000 head per year).

By the statistical analysis, 1), The Before-group-A (dairy cows in Hokkaido which were born before 1995 August, and there was no positive case in BSE test; zero/40,000 tested) and Group-A (they were born from 1995 Sept. to 1996 August, and by the test BSE positive cases were detected; 10/55,000 tested) are different on the BSE prevalence. It suggested that BSE contamination occurred suddenly at the latter half of 1995 in Hokkaido.

2), Statistically Group-A and Post-group-A (they were born from 1996 Sept. to 1999 July, and by the test, zero/250,000 tested) are different on BSE prevalence. It suggested that during 1997 and 1998 there were neither BSE invasion nor propagation in Hokkaido.

3), Statistically Post-group-A and Group-C (they were born from 1999 August to 2001 October, and by the test, BSE positive cases were



detected; 15/170,000 tested) are different on BSE prevalence. It suggested that Group-C outbreak might be induced by BSE propagation in Hokkaido (from Group-A). It is because spatial overlapping of the positive cases in Groups-A and -C was observed, and the interval between Group-A and -C might be consistent with the BSE incubation period. Other than Hokkaido (in Kanto and Kyushu), indigenous propagation of BSE was not detected by the extensive nationwide BSE surveillance until now.

Strangely, however, it is very important that the invasive BSE risk into Hokkaido was very poor. That is, all the UK imported live cattle were rendered in Saga prefecture (Kyushu) and Kanagawa prefecture (Kanto), and consumed as animal feed outside of Hokkaido (mainly in Kanto, and Kyushu). There were no reports of Italian MBM importation into Hokkaido (they were imported into Kanto, Chubu, and Kyushu). The Germany live cattle were rendered in Hokkaido, bat it was occurred later than 1996 (after the A-group outbreak in Hokkaido) and the MBM used in the Hokkaido feed factory from 1995 April, was derived from Hokkaido cattle at that time. Thus it may be impossible that the MBM produced at that time was contaminated by BSE in Hokkaido. On the contrary, all BSE cattle of the A-group drunk the milkreplacer made of Holland animal fat (Hokkaido 10 cases, and Kanto 3 cases).

6. BSE contamination scenario and its contradiction

The hypothesis that animal fat imported from Holland from 1995 to 1996 being a causative agent, is examined by a case control study. The farms of Group-A Hokkaido, Kanto and control cases (population control) were randomly sampled as 200 farms from 20,000 dairy farms in the East Japan where the milk replacer made from Holland animal fat might be used. The randomness of the selected farms was confirmed by statistics. As a result, the concerned milkreplacer shared about 30% in Hokkaido at that time. The case control study resulted in 1% significance on P < 0.0001, odds ratio $= 39.3$, and 95 % intervals (4.9-312.9), suggesting that the hypothesis is statistically significant. The same results was obtained by the comparison of expectation value

BSE outbreak of dairy cow in Hokkaido

Birth before 1995 Aug.	1995 Sep.-96 Aug.	96 Sept.- 99 July.	99 Aug.-01 Oct
Before group A BSE case zero/40,000	Group A BSE cases 10/55,000 (13~16/130,000)	Post group A BSE case Zero/250,000	Group C BSE cases 15/170,000

1, Statistically Before-group-A and Group-A are different on BSE prevalence.
It suggested that contamination occurred suddenly at the latter half of 1995.

2, Statistically Group-A and Post-group-A are different on BSE prevalence.
It suggested that on 1997, 98 there were no BSE invasion in Hokkaido.

3, Statistically Post-group-A and Group-C are different on BSE prevalence.
It suggested that Group-C outbreak might be induced by BSE propagation in Hokkaido (from Group-A)

4, Spatial overlapping of the positive cases in Groups-A and -C was observed, and the interval between Group-A and -C might be consistent with the BSE incubation period.

5, Other than Hokkaido, indigenous propagation of BSE was not detected.

and actual value using milkreplacer of the T factory (Pure milk / Milfood-A-Super made of Holland animal fat) and BSE positive case in Hokkaido. The A-group being independent or not was examined (share of the milkreplacer was in Hokkaido = 30 %), and it was statistically significant ($p = 0.00001$).

In deed, the record of animal fat importation from Holland (shipping days in 1995, 1996) confirmed that the 1st lot was1995 July 15, 2nd lot 1995 September 29, 3rd lot 1995 December 9, and 4th lot 1996 January 29. In the T factory, the milkreplacer was produced from 1996 January and February (Pure milk, and Milfood-A -super) from the 2nd lot which was arrived at 1996 December, and transported to the East of Japan. The Hokkaido A-group dairy cow were born from 96 February to 96 August and in Kanto from 1995 December to 96 March. The maximum term of validity on the milkreplacer may be about 6 months after production. Thus, if 2nd lot was contaminated, all dairy cows might drink the same milkreplacer of this lot.

Thus, the circumstantial evidences suggested that the milkreplacer containing Holland animal fat might be the cause of A-group outbreak in Hokkaido and Kanto on 1995 and 96. The Holland epidemiological study reported on the animal fat as follows. There were possibilities that fatty tissues surrounding intestine including the nervous tissues and the ileum which might be infected with BSE prion, were rendered at that time. There was possibility, when collecting the skull and spinal cord for rendering, the CNS was included in them. It was sure that the SRM removal was not obligated before 1977 and nervous tissues might be included. Rendering fat should be ruled containing less than 0.15% impurities, however, before it was ruled as 0.5%. On the contrary, fancy tallow had less than 0.02% impurities.

If the hypothesis of Holland animal fat as causative material was accepted, there are several unexplainable points. By the EFSA evaluation, one full blown BSE cattle is considered to have an infectivity titer of 4160 CoID50. When one adult cattle is rendered, resultant 65kg BMB may contain 32,8kg protein and 13.5kg yellow grease of 0.5% impurities may contain 33g protein.

Feed history of Group A cases (Hokkaido, Kanto)

case	1-2M (milk replacer)	3-6M (calf starter)	7-12M	>1y
1	Milfood Asup	Milfood B flake	Young cow lead	Young cow lead
2	Milfood Asup, Pure milk H	Milfood B green	New sun lucky18	New sun lucky18
4	Milfood Asup	Milfood Asup	Milfood Asup New step 16	New step 16
6	Milfood Asup	Milfood B	Support 70	Support 70
7	Milk	Milfood Asup Infant green	Infant green	Young cow lead
11	Milfood Asup, Milfood B flake	Support 70	Support 70	Support 70
13	Pure milk H, Milfood B green Infant green	Infant green	Infant green New lead 18	New lead 18
15	Milfood Asup, New step 18 Calf top L	New step 18 Calf top L	New step 18	New step 18
16	Milfood Asup, Milfood B flake	Young cow lead	Young cow lead Tokuhai 18ME	Tokuhai 18ME
19	Milfood Asup	Milfood Asup	Calf meal ,Yodel 18	Yodel 18
3	Pure milk, Moret. premium	Pure milk, premium starte		
5	Milk, Pure milk	Pure milk	Maybypass40	Maybypass 40
10	Pure milk	Pure milk		

Theoretical MBM infectivity by the ordinal rendering method (110℃, 1bar, 60 min) with cross contamination on 1995 in Holland is 2.01 $CoID_{50}$ ($3739 \times 1 \times 0.1 \times 0.005 = 2.01$). On the other hand, theoretical animal fat infectivity at that time in Holland may be 0.37 $CoID_{50}$ ($3.8 \times 1 \times 0.1 \times 0.97 = 0.37$). Therefore, the contaminated milkreplacer lot had to be made of 54〜81 full blown BSE cattle theoretically in order to induce A-group outbreak (Ca. 20 〜30 cases), provided the worst scenario was accepted (impurity of the animal fat was 0.5%, including the central nervous system and other SRM such as ganglion). By the Holland epidemiology, however, such kind of high dose of BSE contamination could not be occurred. Probably there are missed risk factors in this estimation, such as un-homogeneous protein impurity or higher content of SRM in animal fat, and high intestinal absorption of neonatal cattle (< 1 month old) than the calf which are used for the experimental infection (3〜6 months old). Collection of scientific evidences relating these possibilities will be needed.

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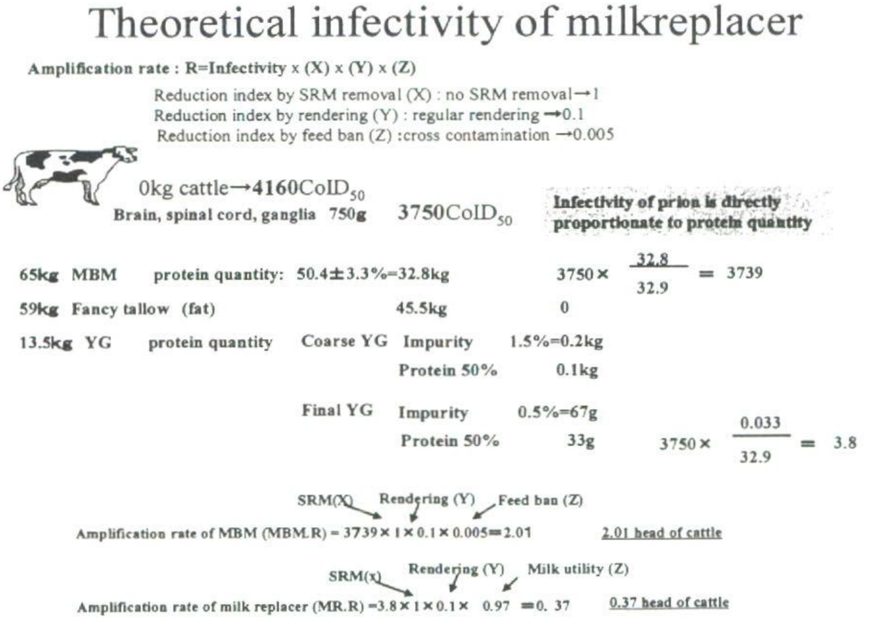

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