

Animal feed controls implemented in Japan for the eradication of bovine spongiform encephalopathy

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Summary

After the detection of the first case of bovine spongiform encephalopathy (BSE) in Japan in September 2001, the Japanese government introduced a series of animal feed control measures to reduce the risk of the spread of the disease from a feed source. To ensure the proper implementation of these measures, the Food and Agricultural Materials Inspection Centre conducted audit inspections of feed importers, producers, distributors and end-users. The audit inspections include on-site inspection of the feed plants, warehouses, farms and other related premises and the laboratory analysis of feed samples taken from these premises to check for the presence of animal protein. The results of inspections conducted in recent years indicate good compliance with the feed control measures.

Keywords

Audit, Bovine spongiform encephalopathy, Control, Feed, Inspection, Japan, Mad cow disease, Meat-and-bone meal.

I controlli sui mangimi animali in Giappone per l'eradicazione dell'encefalopatia spongiforme bovina

Riassunto

Dopo la scoperta del primo caso di encefalopatia spongiforme bovina (BSE) in Giappone nel settembre 2001, il governo giapponese ha introdotto una serie di misure di controllo dei mangimi

animali al fine di ridurre il rischio di diffusione della malattia da questa fonte. Per assicurare la corretta implementazione di queste misure, il Food and Agricultural Materials Inspection Centre ha condotto audit ispettivi sugli importatori di mangimi così come su produttori, distributori e clienti finali. Queste ispezioni prevedono un sopralluogo in impianti, magazzini, fattorie e altre strutture correlate e l'analisi in laboratorio dei campioni di mangimi lì prelevati per indagare l'eventuale presenza della proteina animale. I risultati delle ispezioni condotte negli ultimi anni indicano una buona osservanza delle misure di controllo dei mangimi.

Parole chiave

Audit, Controllo, Encefalopatia spongiforme bovina, Farine ossee, Giappone, Ispezione, Mangime, Morbo della mucca pazza.

Introduction

Bovine spongiform encephalopathy (BSE) is a progressive and fatal disease that affects the central nervous system of cattle. It was first diagnosed in the United Kingdom in 1986. BSE was subsequently reported in several other countries in the 1990s. Meat-and-bone meal (MBM) contaminated with a scrapie-like agent is considered to be the vehicle of BSE infection (12). Cattle that have consumed feed containing MBM contaminated with the BSE agent may become infected and develop clinical signs after a mean incubation period of 4-6 years.

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The Japanese government has taken various measures to protect the cattle population from exposure to contaminated feed, namely:

- the importation of live cattle and MBM has been prohibited from the United Kingdom and other BSE-affected countries (except for MBM heat-treated at 133°C, 3 bar for 20 min) since July 1990
- the importation of MBM from the United Kingdom has been prohibited since March 1996
- an administrative guidance was issued to prohibit the use of ruminant MBM in ruminant feed in April 1996
- the importation of MBM was prohibited from European Union Member States, Switzerland and Liechtenstein in January 2001.

In spite of these measures, the first case of BSE was confirmed in Japan on 10 September 2001. After the detection of the first case, the Japanese government introduced a series of feed control measures to prevent the spread of BSE. As a result, the epidemic peaked in 2006 and no case has been detected in cattle born after January 2002 among 34 additional cases that were detected by the end of January 2009.

This paper describes the evolution of feed controls taken for the prevention of BSE and the audit inspection and analysis of feed samples to ensure the proper implementation of feed controls.

Evolution of feed controls to prevent bovine spongiform encephalopathy in Japan

The Japanese government has taken, among many actions, various feed control measures to protect the cattle population from exposure to feed potentially contaminated with the BSE agent. A voluntary feed ban was introduced in 1996 in the form of an administrative guidance, as described below.

Administrative guidance on the uses of meat-and-bone meal in April 1996

An administrative guidance was issued by the Ministry of Agriculture, Forestry and Fisheries

(MAFF) on 16 April 1996 calling for feed producers to withdraw MBM from the production of compound feed for ruminants. This administrative guidance was successful in decreasing the use of MBM as an ingredient of compound feed for cattle. Between 1989 and 1995, the amount of MBM used each year as raw material for the production of cattle compound feed was 83 to 247 metric tons (13). This amount decreased to zero after the administrative guidance was issued. However, this administrative guidance was not sufficient to prevent cross-contamination of cattle feed in plants where feed for cattle, pigs and chickens were produced using a same production line. The Japanese government was severely criticised later for not installing a compulsory feed ban at this time.

Guidelines for the Prevention of Cross-contamination of Feed with Ruminant Proteins, June 2001

On 1 June 2001, Guidelines for the Prevention of Cross-contamination of Feed for Ruminants with Ruminant Proteins were issued by the MAFF. These provide the basic rules to follow to prevent cross-contamination of feed with animal proteins at all stages of production, importation, distribution and use of feed.

Ministerial order banning ruminant protein in feed, September 2001

On 18 September 2001 (8 days after the detection of the first case of BSE), the MAFF introduced a compulsory ban on the domestic use of ruminant protein for ruminant feed. This was done by amending the ministerial order issued under the Feed Safety Law.

Ministerial order banning all processed animal proteins in feed, October 2001

On 15 October 2001, the ministerial order was amended to introduce a 'complete feed ban' which prohibited the domestic use and importation of all processed animal proteins (mammal, poultry and fish protein, excluding milk protein) for the production of feed for ruminants, pigs and chickens and for use as fertilizer.

Administrative guidance and ministerial order on the use of animal fat

On 27 December 2001, the MAFF issued an administrative guidance calling for the suspension of the use of animal fat with insoluble impurities over 0.15% for animal feed, effective on the same date. The guidance also requested the suspension of the use of animal fat with insoluble impurities over 0.02% for milk replacers, effective from 15 January 2002. This provision became compulsory when an amended ministerial order was issued on 2 August 2002.

Guidelines for the Prevention of Cross-contamination of Feed with Animal Proteins, September 2003

On 16 September 2003, Guidelines for the Prevention of Cross-contamination of Feed with Animal Proteins were issued by the MAFF. These provide the basic rules that should be followed to prevent cross-contamination of feed with animal proteins at each stage of production, importation, distribution and use of feed (6).

Ministerial order banning fish meal in feed, January 2004

On 15 January 2004, the ministerial order issued in October 2001 was amended again to introduce an additional ban on the use of fish meal for feed except for that produced in a designated plant where measures are taken to prevent cross-contamination.

Ministerial order banning vertebral columns and fallen stock, May 2004

On 1 May 2004, the ministerial order was reinforced to ban the use of bovine vertebral columns and fallen stock for production of animal fat, and the use of ruminant animal fat for cattle feed.

Ministerial order exempting swine and poultry proteins from feed ban, April 2005

On 1 April 2005, the feed ban was altered to allow the use of proteins derived from swine and poultry in feed for swine and/or poultry

on condition that these proteins were produced in designated facilities where no ruminant materials were handled. On this date, the Guidelines for the Prevention of Cross-contamination of Feed with Animal Proteins were amended accordingly (6).

Amendment of the Feed Safety Law Enforcement Regulation, June 2005

On 30 June 2005, the Feed Safety Law Enforcement Regulation was amended so that all importers of feed and feed ingredients must report the nature of imported feed and feed ingredients to the MAFF.

Ministerial order exempting swine and poultry proteins from feed ban, May 2008

On 28 May 2008, the feed ban was amended to allow the use of proteins derived from swine and poultry in feed for aquatic animals on condition that these proteins were produced in designated facilities where no ruminant materials were handled.

Table I shows the status of regulations on the use of processed animal proteins and animal fat as of 1 May 2008. Figure 1 illustrates the various feed control measures taken to prevent the spread of BSE and the organisations involved in implementing these measures.

Audit inspections of feed

Audit inspections of feed producers with feed plants located nationwide are conducted by the Food and Agricultural Materials Inspection Centre (FAMIC) under instruction from the MAFF. Audit inspections of feed distributors operating locally and end-users of feed (cattle farmers) are conducted by prefecture governments. Feed samples are collected at importers' warehouses, feed plants and distributors' warehouses. The officials of the FAMIC and prefecture governments are granted the right to make on-site inspections and take feed samples under the Feed Safety Law. On-site inspections are conducted in accordance with the instructions issued by the MAFF in October 2005 (7) to ensure that the Guidelines for the Prevention of Cross-

Table I
Regulations on the use of processed animal proteins and animal fat

Types of animal protein and animal fat	Ruminants	Pigs	Feed for Poultry	Cultured fish
Processed animal proteins				
Gelatine and collagen of mammalian origin ^(a)	A	A	A	A
Milk and dairy products of mammalian origin	A	A	A	A
Eggs and egg products	A	A	A	A
Blood meal and plasma meal of bovine origin	B	B	B	B
Blood meal and plasma meal of pig, horse and/or poultry origin	B	A	A	A
Fish meal and other proteins of fish origin ^(a)	B	A	A	A
Poultry meal and feather meal ^(a)	B	A	A	A
Hydrolysed protein and steamed bone meal of poultry origin ^(a)	B	A	A	A
Meat-and-bone meal, hydrolysed protein and steamed bone meal of pig and/or poultry origin ^(a)	B	A	A	A
Meat-and-bone meal, hydrolysed protein and steamed bone meal of ruminant origin	B	B	B	B
Animal fat				
Animal fat produced from edible fat containing insoluble impurities of 0.02% or less	A	A	A	A
Animal fat of bovine origin containing insoluble impurities of 0.15% or less ^(a)	B	A	A	A
Animal fat of non-bovine origin containing insoluble impurities of 0.15% or less ^(a)	A ^(b)	A	A	A
Fish oil produced at a plant where animal protein other than fish is not handled	A	A	A	A

A permitted

B banned

(a) those that are processed at a plant approved by the Minister of Agriculture, Forestry and Fisheries

(b) banned for use in milk replacers

contamination of Feed with Animal Proteins are observed.

Audit inspections of feed importers

In regard to imported feed, the MAFF ensures that no animal protein is used by checking the importers list of used ingredients. In addition, 35, 43 and 31 samples of imported mixed feed and feed additives for ruminant were taken in fiscal years 2005, 2006 and 2007, respectively, with no animal protein detected (8, 9, 11). Every consignment of imported fish meal is inspected by the animal quarantine service for the presence of animal proteins and to ensure that it is from a designated plant where no mammalian or poultry materials are handled.

Audit inspections of feed producers

At the end of 2007, there were 2 451 plants producing feed and/or feed additives. In fiscal

years 2006 and 2007, 759 and 569 plants received on-site inspections by the FAMIC and prefecture governments, respectively (9, 11). The results of these inspections are given in Table II. During the on-site inspections, the officials of the FAMIC or prefecture governments check production records and production lines, warehouses and other facilities to ensure that:

- no prohibited materials are used
- the Guidelines for the Prevention of Cross-contamination of Feed with Animal Proteins are observed
- a feed quality management manual is available and used
- the target animals are specified on the labels according to the labelling standards
- the packages or containers for feed for non-ruminants are labelled 'Not for use in ruminants'.

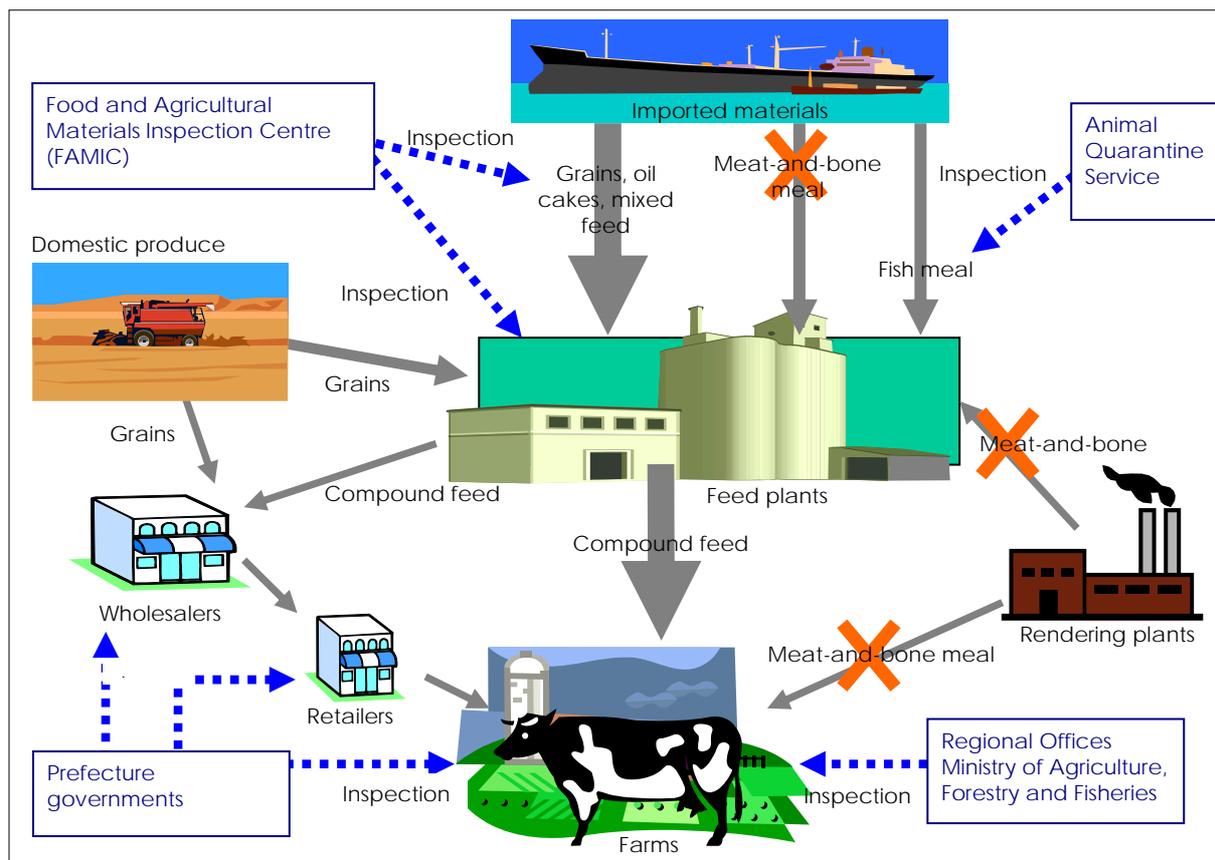


Figure 1
Animal feed control measures taken to prevent the spread of bovine spongiform encephalopathy showing the organisations involved in implementing these measures
Grey arrows indicate the movements of feed and feed ingredients
The crosses indicate prohibited materials, dotted arrows indicate inspections

Table II
Number of violations of the Guidelines for the Prevention of Cross-contamination of Feed with Animal Proteins in feed producers in fiscal years 2006 and 2007

Types of violations	2006	2007
Improper maintenance of sales records	3/759	4/569
Improper labelling	13/759	3/569
Improper separation of production lines	1/759	1/569
Improper separation of feed in storage and transportation	1/759	1/569

Numerators indicate the number of plants with a reported violation and denominators the number of plants inspected

Audit inspections of feed distributors

At the end of 2007, there were 13 080 feed and feed additive distributors. In fiscal years 2005, 2006 and 2007, 331, 1 464 and 675 distributors, respectively, were selected for on-site inspections by prefecture government officials

(8, 11, 13). The results of these inspections are shown in Table III. During the on-site inspections, the prefecture government officials check the sales records, warehouses, transport vehicles and other facilities to ensure that:

- the Guidelines for the Prevention of Cross-contamination of Feed with Animal Proteins are observed at each stage of reception, storage, shipping and transportation of feed
- a feed handling manual is available and used
- feed for pigs and poultry is not sold to farmers keeping ruminants
- containers used for ruminant feed are used for ruminant feed only
- there is no feed with improper labelling.

Audit inspections of feed end-users (farmers)

By February 2008, there were 97 293 farms on which cattle were raised in Japan. In fiscal

years 2005, 2006 and 2007, 1 585, 6 244 and 8 310 farms, respectively, were visited for on-site inspection by prefecture government officials (8, 9, 11). The results of these inspections are shown in Table IV. During these on-site inspections, the prefecture government officials checked feeding records, warehouses, animal houses and other facilities to ensure that:

- the Guidelines for the Prevention of Cross-contamination of Feed with Animal Proteins are observed at each stage of reception, storage and use of feed
- records are kept on the feed used
- there is no feed with improper labelling.

Table III
Number of violations of the Guidelines for the Prevention of Cross-contamination of Feed with Animal Proteins in feed distributors in fiscal years 2005, 2006 and 2007

Types of violations	2005	2006	2007
Improper maintenance of sales records	14/331	2/1 464	25/675
Improper labelling	0/331	1/1 464	0/675
Improper separation of feed in storage and transportation	5/331	4/1 464	17/675

Numerators indicate the number of distributors with a reported violation and denominators the number of distributors inspected

Analysis of feed and feed ingredients

Feed samples taken at importers' warehouses, feed plants and distributors' warehouses are sent to the FAMIC for detection of prohibited materials.

Techniques used

To detect the presence of prohibited materials (animal proteins), animal feed samples are examined by microscopy, enzyme-linked immunosorbent assay (ELISA) and polymerase chain reaction (PCR), following the procedures prescribed in the *Detection Method of Animal Proteins in Feed* (10). The ELISA and PCR methods were validated for sensitivity and specificity for the presence of animal proteins in feed. Collaborative studies were conducted

with the participation of 10-20 laboratories in accordance with the criteria of the official method analysis of the Association of Official Analytical Chemists (AOAC). These methods are registered in the Official Method Feed Analysis of Japan (10).

Table IV
Number of violations of the Guidelines for the Prevention of Cross-contamination of Feed with Animal Proteins in end-users of feed (cattle farmers) in fiscal years 2005, 2006 and 2007

Types of violations	2005	2006	2007
Improper separation of feed in storage and transportation	25/1 585	7/6 244	4/8 310

Numerators indicate the number of end-users (cattle farmers) with a reported violation and denominators the number of end-users (cattle farmers) inspected

Microscopic method

Compound feed and feed ingredient samples collected by the on-site inspectors are subjected to the microscopic examination for the presence of cartilage, bone, horn, hair, bristles, blood, feather, egg shells, fish bone and scales.

The microscopic method is especially effective for the detection of bone tissue which is treated at high temperatures during the rendering process. This method can detect mammalian, poultry, and fish bones, but cannot distinguish between animal species, e.g. cattle and pig bones. To detect ruminant protein in the presence of pig MBM, one must resort to the ELISA and PCR methods.

Enzyme-linked immunosorbent assay

Compound feed for cattle and feed materials of animal origin (e.g. chicken meal, feather meal, fish meal, pork meal) are subjected to an ELISA. Three ELISA kits are used for the detection of heat-treated animal proteins, as follows:

- Bovine protein and poultry protein in compound feed and fish meal are tested with the Morinaga® kit. This kit uses species-specific polyclonal antibodies against bovine (or poultry) serum albumin and can detect various cattle (or poultry) tissues such as muscle, blood, milk, and gelatine made from bone or skin.

- The presence of ruminant protein in MBM of poultry and pig origin is tested with the Melisa-Tek® ruminant kit. This kit uses monoclonal antibodies against heat-stable muscle protein. It can detect prohibited ruminant materials, such as MBM, without cross-reacting to permitted bovine-derived materials such as milk.
- Porcine protein in feed materials of animal origin can be detected using the ELISA-Tek® pork species kit.

A positive result is confirmed by additional testing using PCR to detect mammalian DNA and species-specific DNAs.

Polymerase chain reaction

All samples of compound feed for ruminant and feed ingredients are subjected to PCR for detection of mammalian mitochondrial DNA (mtDNA) or ruminant mtDNA. PCR is most effective for the detection of small amounts of MBM in ruminant feed and for the identification of species of origin. The official PCR method uses PCR primer sets for the detection of specific species (e.g. bovine, porcine, ovine) and multiple species (e.g. mammals, ruminants, poultry, fish). When mammalian DNA or ruminant DNA is detected in feed samples by PCR, they are additionally tested by PCR to identify the species of origin. To detect bovine MBM in milk replacers and other feed samples containing dairy products, the dairy products are removed before subjecting the samples to the PCR.

Table V shows the characteristics of the three methods used for the detection of animal proteins. Tables VI A and VI B give the judgment trees for the presence or absence of prohibited animal proteins using the three detection methods. The judgment for the presence or absence of prohibited animal proteins in ruminant feed depends on the results of the three methods (Table VI A). Determining the presence or absence of prohibited animal proteins in feed ingredients for pigs, poultry and fish depends on the results of ELISA and PCR (Table VI B), because microscopic methods are not effective to detect ruminant protein in feed ingredients that also contain protein of pig and/or poultry origin.

Results of analysis

Table VII provides the number of samples subjected to laboratory analysis for the presence of animal protein and DNA in the last five years. No mammalian proteins have been found in chicken meal and feather meal samples since fiscal year 2003 (1, 2, 3, 4, 5).

Overall, 0-4 fish meal samples (0-3.8%) are found positive annually for the presence of poultry proteins. The subsequent investigation of the fish meal plants revealed that accidental mixing of feathers with imported fish meal or poultry meat with waste scraps used as raw materials for fish meal was suspected to be responsible for the presence of the poultry protein.

Table V
Characteristics of official methods used in Japan for the detection of animal tissues in feed

Performance indicator	Microscopy	ELISA	PCR
Target of detection	Cartilage, bone, horn, hair, bristles, blood, feather, egg shells, fish bone and scales	Animal protein	Animal DNA
Sensitivity of detection (detection limit in terms of MBM in Feed (w/w)%)	Not high (0.1~0.3 %)	Not high (0.1~1 %)	High (0.01~0.1 %)
Accuracy of discernment for animal species	Low	Medium	High
Accuracy of discernment for animal tissue	High	Medium	Low

ELISA enzyme-linked immunosorbent assay
PCR polymerase chain reaction
MBM meat-and-bone meal

Table VI A
Judgment tree used by the Food and Agricultural Materials Inspection Centre in Japan for the detection of prohibited protein in ruminant feed

Test and result			Final judgment
Microscopy	ELISA ^(a)	PCR ^(b)	
Positive	Positive or negative	Positive or negative	[Prohibited protein present]
Negative	Positive	Positive	
		Negative	[Prohibited protein absent]
	Negative	Positive or negative	

ELISA enzyme-linked immunosorbent assay
PCR polymerase chain reaction
(a) ELISA for the detection of ruminant, pig and poultry protein
(b) PCR for the detection of ruminant, pig and poultry DNA

Table VI B
Judgment tree used by the Food and Agricultural Materials Inspection Centre in Japan for the detection of prohibited protein in feed ingredients for pigs, poultry and fish

Test and result		Final judgment
ELISA ^(a)	PCR ^(b)	
Positive	Positive	[Prohibited protein present]
	Negative	[Prohibited protein absent]
Negative	Positive or negative	

ELISA enzyme-linked immunosorbent assay
PCR polymerase chain reaction
(a) ELISA for the detection of ruminant protein
(b) PCR for the detection of ruminant DNA

Table VII
Number of feed samples collected and analysed by the Food and Agricultural Materials Inspection Centre in Japan for the presence of animal protein in fiscal years 2001-2006

Types of feed	2001	2002	2003	2004	2005	2006
Chicken meal	0/0	2/25	0/32	0/29	0/35	0/31
Feather meal	0/0	0/17	0/21	0/21	0/23	0/23
Fish meal	4/94	1/106	0/129	2/113	4/113	0/107
Compound feed for cattle	0/0	7/112	3/184	6/216	0/216	0/185
Pig meat-and-bone meal ^(a)	0/0	0/0	0/0	0/0	0/8	1/27
Other feed and feed materials ^(b)	0/0	0/3	0/5	0/8	0/7	0/11

(a) pig meat-and-bone meal samples were tested for presence of ruminant protein.
(b) other feed and feed materials include mixed feed of fish meal and oil cakes, mixed feed of feather meal and oil cakes, hydrolysed proteins, etc.

Numerators indicate the number of samples with positive results, and denominators the number of samples tested

Up until fiscal year 2004, 3-7 compound feed samples (1.6-6.3%) were found positive for presence of ruminant protein annually. Feed plants with positive results were revisited for inspection of production lines. As a result of these inspections, skimmed milk powder and whey powder were found to be used as raw materials and responsible for these positive results.

Conclusions

Effective controls on animal feed are the key to the elimination of BSE derived from a feed source. The administrative guidance on feed, introduced in 1996, was not totally effective in preventing the exposure of the Japanese cattle population to BSE-contaminated feed. However, the complete feed ban, introduced in

October 2001 in accordance with the Feed Safety Law, appears to be effective. The results of on-site inspections of feed importers, producers, distributors and end users (farmers) indicate good compliance with the feed regulations.

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