# Regulatory Frameworks to ensure Feeds Safety in Japan

**Food and Agricultural Materials Inspection Center**

**(FAMIC)**

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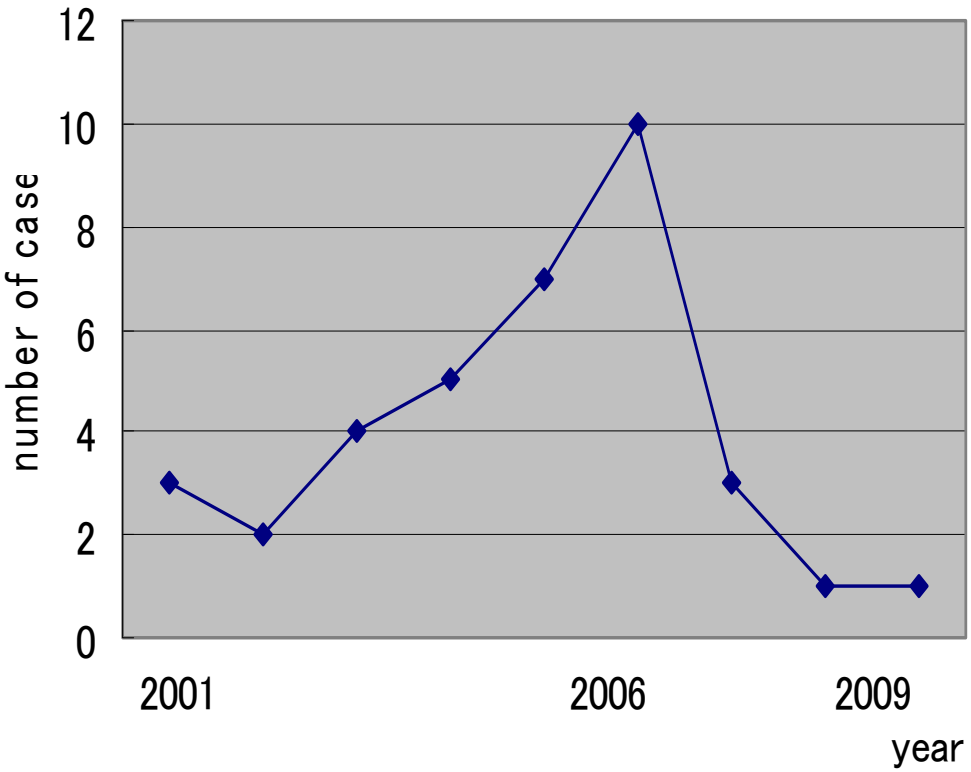
" Law concerning safety assurance and quality improvement of feeds"

# Current situation of occurrence BSE in Japan



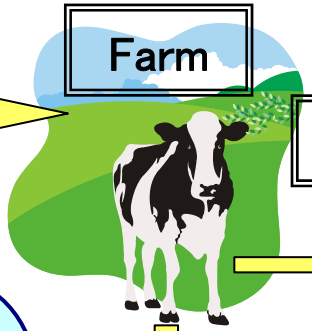

| Year | No. of case | origin | |
|---|---|---|---|
| | | Died cow | Slaughter house |
| 2001 | 3 | 0 | 3 |
| 2002 | 2 | 0 | 2 |
| 2003 | 4 | 0 | 4 |
| 2004 | 5 | 2 | 3 |
| 2005 | 7 | 4 | 3 |
| 2006 | 10 | 4 | 6 |
| 2007 | 3 | 2 | 1 |
| 2008 | 1 | 1 | 0 |
| 2009 | 1 | 1 | 0 |

# BSE-control measures in Japan

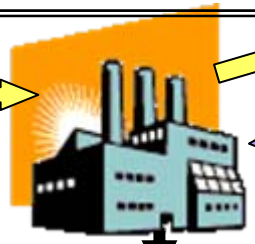
・BSE inspection of all cow (2001,10)
・BSE inspection of all cow for meat older than 21 month-old (2005,8)
Positive cases 22 / Inspection cases 8,930,000 (2008,12)

Meat-and-bone (MBM)

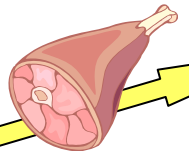
Farm



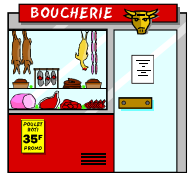
Slaughter House



Burn disposal of SRM



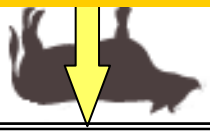
Shop



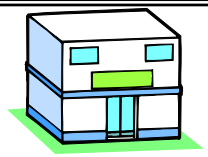

・SRM remove (head except tongue and cheek, spinal cord, ileum ) (2004,12)
・Prohibition of using spine as material of oil and fat (2004,12)

・Prohibition of imports of MBM from all countries (2001,10)
・Prohibition using MBM for manufacture, feed and sale(2001,10)
・Reopen of using MBM originated porcine (licensed by MAFF for prevention of cross contaminations

Died cow



Prefecture Animal Hygiene Laboratory



Burn disposal of died cow

・Obligation of report about the died cow older than 24 month-old in farm and BSE inspection (2003,4)
・BSE inspection of all died cow (2004,4)

# Feed control

Food and Agricultural Materials Inspection Center ( FAMIC )


Animal Quarantine Service

Importers

Grains
Oil cakes
Mixed feed

MBM

Fish Meal

Spot Inspection

Inspection

Domestic production

Grains

Grains

Feed Plants( domestic or importers)

MBM

Compound Feeds

Wholesalers

Compound Feeds

Retailers

MBM

Rendering Plants

Inspection

Farm

Prefecture Governments

Inspection

Inspection

MAFF Regional Offices

# < Current Situation for Feed Ban in Japan > (Animal protein)

| Materials | Origin of materials | Livestock | | | |
|---|---|---|---|---|---|
| | | Ruminant | Swine | Poultry | Fish |
| Gelatin, Collagen (Licensed) | Mammal | ○ | ○ | ○ | ○ |
| Milk, Dairy products | | ○ | ○ | ○ | ○ |
| Egg, Egg product | Poultry | | | | |
| Powdered blood, Plasma | Ruminant | × | × | × | × |
| | Swine, Horse, and Poultry (Licensed) | × | ○ | ○ | ○ |
| Fish meal (Licensed) | Fish and shellfishes | | ○ | ○ | ○ |
| Chicken meal, Feather meal (Licensed) | Poultry | | ○ | ○ | ○ |
| Hydrolysis protein, Steamed bone meal (Licensed) | Poultry | × | ○ | ○ | ○ |
| Meat and bone meal, Hydrolysis protein, Steamed bone meal | Swine (Licensed) | | ○ | ○ | ○ |
| | Swine–Poultry mixture (Licensed) | | ○ | ○ | ○ |
| | Ruminant | × | × | × | × |
| Food industrial wastes including animal protein (food waste etc.) | Mammal, Poultry, Fish and shellfishes | × | ○ | ○ | × |

# <Current Situation for Feed Ban in Japan> animal oil and fat

| Materials | | Standard of insoluble impurities content (less than %) | For cattle | | For swine | For poultry | For fish |
|---|---|---|---|---|---|---|---|
| | | | Substitution milk | Others | | | |
| Specific animal oil and fat | | 0.02 | ○ | ○ | ○ | | |
| Tallow | | 0.15 | × | × | ○ | | |
| | originated from swine and poultry | 0.15 | × | ○ | ○ | | |
| The spinal column and the dead cattle origin of a cow | | | × | × | × | | |
| Recovery cooking oil (Used cooking oil) | | 0.02 | ○ | ○ | ○ | | |
| | | 0.15 | × | × | ○ | | |
| Oil | Fish Oil | - | ○ | ○ | ○ | ○ | ○ |
| | Vegetable Oil | - | ○ | ○ | ○ | ○ | ○ |

# Detection analysis of MBM in Feeds

1) Microscope
2) ELISA analysis for Animal derived protein
3) PCR analysis for Animal derived DNA

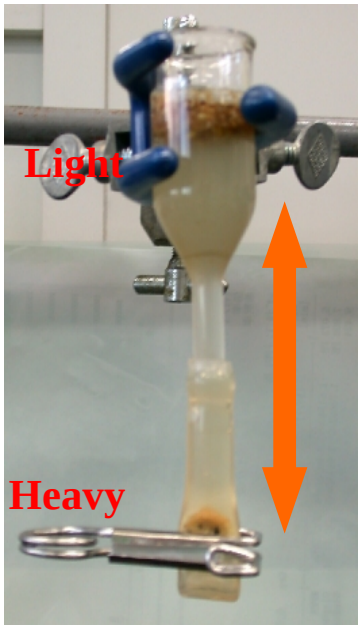
# Summary of three methods for detection of animal proteins in feed

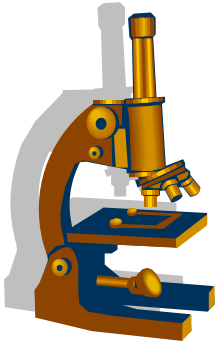
| Method | Heat Stability | Utility | Specificity | | Quantity | Cost performance | Sensitivity (content) |
|---|---|---|---|---|---|---|---|
| | | | Tissues | Species | | | |
| Micro-scopy | ++++ | ++ | ++++ | +/− | − | ++++ | 0.1～0.3% |
| ELISA | ++ | +++ | +/− | +++ | ++ | +++ | 0.1～1 % |
| PCR | + | ++ | − | ++++ | + | ++ | 0.01～0.1 % |

−:not effective; ＋:effective ( Journal of Food Protection,Vol.63,No.11,2000,Pages1602-1609)

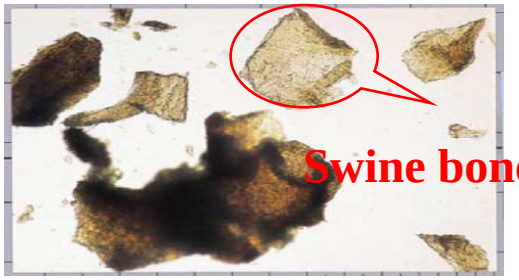
# ＜Microscopy＞

cartilage, bone, horn, hair, bristles, blood, feather, egg shells, fish bone and scales

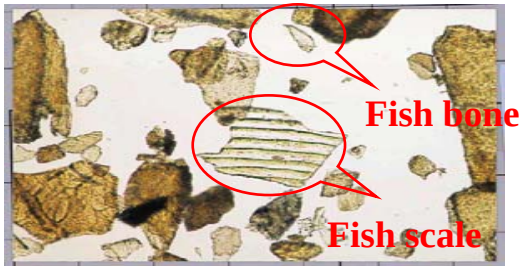


Weigh sample (1g)
↓
Sedimentation　Zinc chloride solution (sp：1.8)
↓
Separation to beaker
↓←add 3%(v/v)$H_2SO_4$
Decantation with water 2 or 3 times
↓←add 5%(w/v)NaOH
Boiling 15 min
↓
Decantation with water 2 or 3 times
↓
Transfer to laboratory dish
(Observation)　x100







# < ELISA> Comparison of ELISA kits

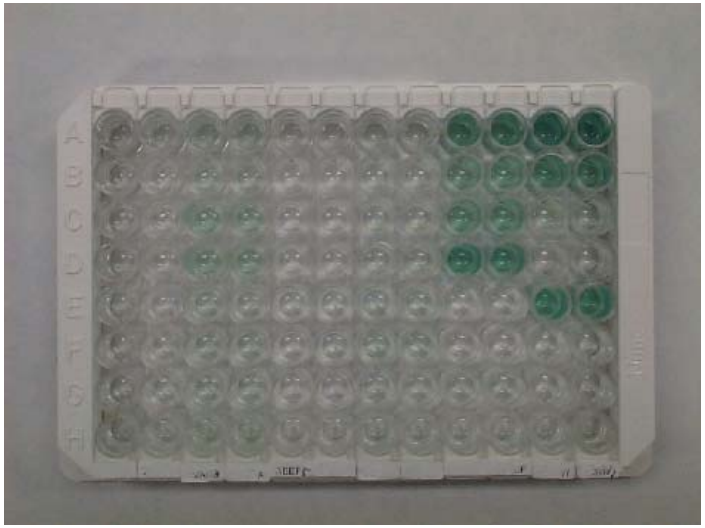
| | ELISA-TEK® pork species kit | MORINAGA® kit | MELISA-TEK® ruminant kit |
|---|---|---|---|
| Manufacturer | ELISA Technologies Inc. | Morinaga Institute of Biological Science, Inc. | ELISA Tecnologies Inc. |
| Object | Fish meal<br>Chicken meal<br>Feather meal<br>Blood powder | Compound feed for bovine, Fish meal | Pork meal<br>Chicken meal<br>Feather meal<br>Mix MBM for swine and chicken |
| Target for detection | Bovine, Swine, Chicken, Sheep/Goat, Deer | Bovine, Chicken | Ruminant |
| Specificity | 0.5-1 % | 0.1-0.5 % | 0.1-0.5 % |
| Tissue | Muscle, Blood, Milk, Gelatin | Muscle, Blood, Milk, Gelatin | Muscle |

## MORINAGA® kit

For detection of bovine protein in compound feed

## MELISA-TEK® ruminant kit

For detection of bovine MBM in swine and chicken MBM (Identification Test)



**Color development**

**Green : Positive**

# < DNA Analysis >

**・For high sensitive detection of a very small amount contaminated MBM and for discrimination of animal species**

Target of PCR : Mitochondrion gene

Characteristic
- ・Large quantities of copy numbers
- ・heat-stable ( suitable for heat-treated materials )
- ・Primers constructed in the animal-specific-region and not exist the plant gene

**possible high specific detection for animal originated DNA**

Sample : Compound feed , Feed materials (MBM, Fish-meal)

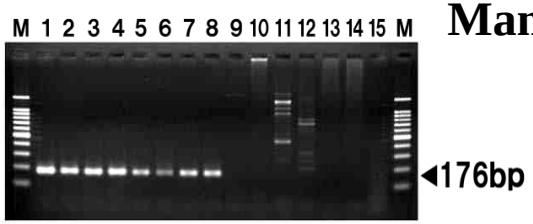
Sensitivity : 0.1g MBM / 1 kg of feed

# < Primer pairs of PCR >

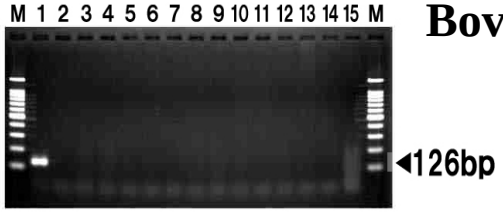
**All primer pairs get the patent in Japan**

| Object of detection | Name of primer | size of amplification | Detectible animal species |
|---|---|---|---|
| Mammalia | anicon 5<br>anicon 3 | 176 | bovine, swine, sheep, goat, deer, rabbit, whale |
| Ruminants | rumicon52<br>rerumicon32 | 201 | bovine, sheep, goat, deer |
| Bovine | cow52<br>cow31 | 126 | bovine |
| Chicken | chick5-1<br>chick3-1 | 133 | chicken, quail |
| Swine | pig-5-6<br>pig3-6 | 83 | swine |
| Fish | FM5<br>FM3 | 78 | anchovy, skipjack, salmon, mackerel, saury, sea bass, sea bream, codfish, pilchard, tuna, |
| Plant | placon5<br>placon3 | 140 | corn, rice, soybean, beet, wheat |

**Mammalian**




**Bovine**




1: Bovine 2: Sheep 3: Goat 4: Deer 5: Swine 6: House 7: Rabbit 8: Whale 9: Chicken 10: Pollock

11: Salmon 12:Sardine 13: Crab 14: Shrimp 15: Clam M: Marker

# < How to do the PCR analyze >

Sample

**compounded feed, feed materials**

Break into pieces (about 1 mm )

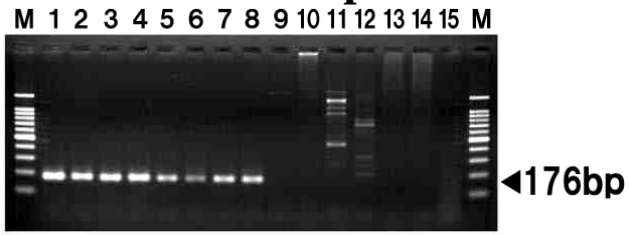
Extraction of DNA

PCR amplification by Mammals primer pairs

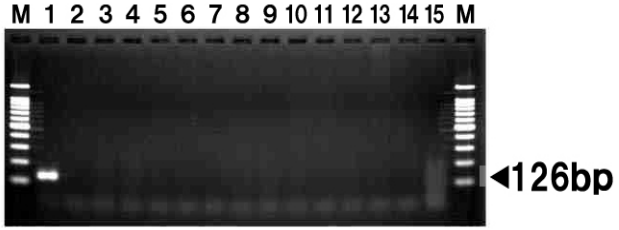
or Ruminants primer pairs

< Screening Test >

**Mammalian primer**




**Bovine primer**




Positive

**Negative**

Confirmation PCR assay

using the species specific primer pairs

# Total Judgment of analysis for animal protein or DNA

| Microscopy | ELISA | PCR | Total Judgment |
|---|---|---|---|
| + | — | — | Positive |
| + | + | — | Positive |
| + | — | + | Positive |
| — | + | + | Positive |
| — | — | + | False-positive |
| — | + | — | Negative (non-specific reaction) |
| — | — | — | Negative |

+ : Positive ( Detective )　　— : Negative ( Non-detective)

**Positive and Pseudo positive sample**
**☆Investigation of cause and Re-test**

the introduce condition of feed materials
the manufacturing process and the distribution process

# The number of the violation cases about MBM contamination in feed

| Types | 2002 | 2003 | 2004 | 2005 | 2006 |
|---|---|---|---|---|---|
| Compound feed for cows | 7/112 | 3/184 | 6/216 | 0/216 | 0/185 |
| Pig MBM | 0/0 | 0/0 | 0/0 | 0/8 | 1/27 |
| Chicken meal | 2/25 | 0/32 | 0/29 | 0/35 | 0/31 |
| Feather meal | 0/27 | 0/21 | 0/21 | 0/23 | 0/23 |
| Fish meal | 1/106 | 0/129 | 2/113 | 4/113 | 0/107 |
| Other feed and feed materials | 0/3 | 0/5 | 0/8 | 0/7 | 0/11 |

( Positive case numbers / sample numbers )

# Thank you for listening

**For Safety and Security of Feeds**