TAFS/FAO/OIE Workshop on Paratuberculosis/MaP November 26 – 28, 2007 Unterägeri, Switzerland

SUMMARY OF RESULTS

Bern, February, 2008

Executive summary

Bovine paratuberculosis/MaP causes severe economic losses and is a potential cause of concern in terms of food safety as long as its zoonotic nature cannot be ruled out. A large set of measures to control the disease and to reduce the bacterial load in food has been introduced in different production systems. Most of them share the advantage that they will be effective against other pathogens as well, while their major disadvantages are that they represent substantial additional cost factors and that there are few quantitative data concerning their effectiveness. Still, control of the disease and of the contamination of food with is possible, provided that the most adequate measures are selected for the specific circumstances.
Research needs requiring immediate attention include better, i.e. more sensitive and more specific diagnostic tests, particularly for young animals, as well as a better understanding of the quantitative effectiveness of disease control and pathogen inactivation measures.

Contents

1 Objectives

The Swiss foundation TAFS (Transmissible Animal Diseases and Food Safety), in collaboration with FAO and OIE, organized a 2 ½ day workshop on bovine Paratuberculosis (pTB) / Johne's disease (JD) and its etiological agent (*Mycobacterium avium paratuberculosis* –MaP).
The objectives of the workshop were:

- To present facts about JD as an animal health issue in order to increase the awareness on the current knowledge and its gaps about the disease, particularly diagnostic tools, epidemiology and risk management in animal populations.

- To determine the gaps along the food chain from a risk management perspective (both by public and private sectors) on possible food safety risks related to JD.

- To discuss and possibly identify a consensus among the participants on the needs to prevent and control paratuberculosis in animals and to continue research in this field.

2 Results

2.1 ANIMAL LEVEL

Primary Principles

Confirming the herd infection status is always the first step, since there is no point in advocating control unless you first know the herd is MaP-infected.

This confirmation is only possible by culture or PCR (organism detection-based test). Control programmes are always based on a combination of management / hygiene measures and detection of infected animals using a variety of diagnostic tools.

However, such hygiene programs are generic and preventative and have multiple benefits even in non-MAP infected herds.

Doing something reliable to control pTB is better than doing nothing.

pTB control is feasible with existing tools.

- Supporting data are available from various countries in different production types of herds, but mainly in dairy herds.
- It takes several years of annual testing to declare a herd which is not MaP infected free of the disease.
- Therefore, eradicating the disease from a farm that is infected will take many years: focus should be on control to prevent disappointment.
- At the start of a control program, the aim should be to remove the heavy shedders in order to reduce the infection pressure on the farm and to reduce the contamination of the milk supply.

Control by animal husbandry (hygiene) improvement has multiple animal health & welfare benefits.

- It controls many fecal-oral pathogens simultaneously.
- It moves farmers toward “best farming practices”.

On-farm control programs should be simple and based on common sense.

- Easy to explain and to sell to the producers.
- No promises of quick results should be made.
- Limited room for argument by experts.
- Requires training of vets (or others) guiding the farmers.

On-farm control program should be at least cost.

- Money matters!
- Limits resistance by producers.
- Balances cost of control against cost of disease.

Potential disease control points

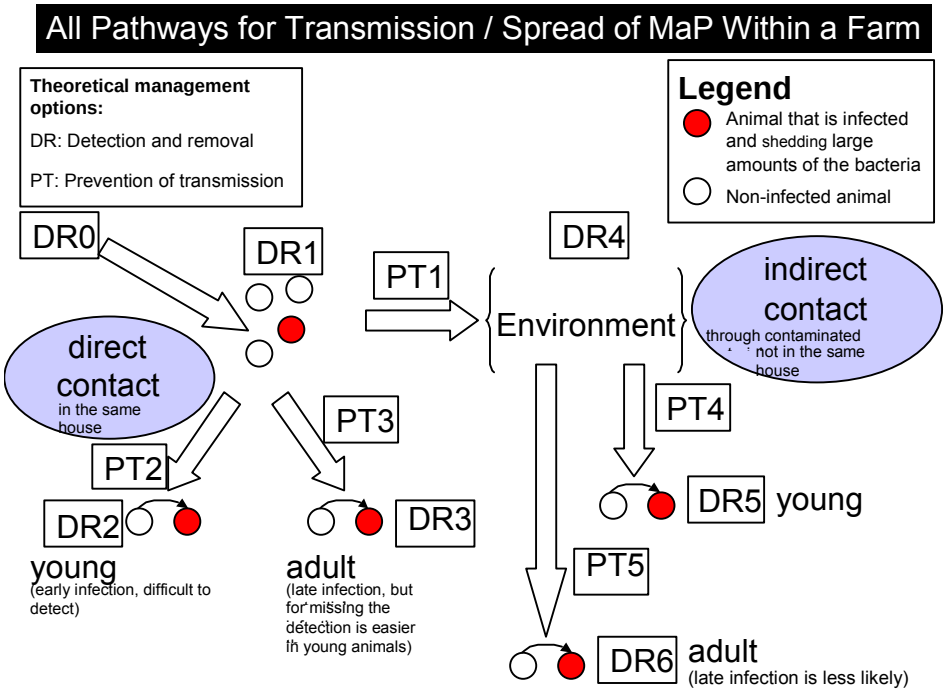

Exhibit 1: Disease control points on farm level

The most important control points are DR0, DR1, and PT2.

DR0 and DR1

- Description:
  - Herd additions should mainly be based on herd-level laboratory tests performed on the herd of origin, and/or on the individual animal and therefore requires a classification / certification protocol to classify herds of origin into high/low risk or free herds.
  - The more animals tested per herd the greater the confidence in the herd's infection status will be. However, false positive outcomes may be increased with more animals tested and positive test results will need confirmation by more specific test (e.g., culture) depending on the consequences of the positive outcome.
  - Repeated annual negative herd tests will increase the confidence in the true non-infected status of the herd.

- Some countries have operational plans for herd classification (certification).

- Principles
  - It is economically senseless to buy MaP-infected cows and then spend money to detect them by laboratory tests them for removal (DR1).
  - The risk of introducing MaP-infected cows can not be reduced to zero (with currently available diagnostic tests).

- (Repeated) herd-level testing can classify herds as low risk.

- Advantages:
  - Multiple program plans are available, e.g. Denmark, Netherlands, Australia, USA.
  - Assist in limiting further MaP infection spread among herds and contamination of the milk supply.
  - Preventive measures of infection or disease are always selected for cost-effectiveness after herds are already infected.

- Disadvantages:
  - Added cost of doing business.
  - In most developed countries there are only few herds not infected with MaP.
  - It requires sufficient laboratory capacity and trained personnel to handle large sample numbers.

- Conditions for effectiveness:
  - Access to accurate and affordable diagnostic tests.
  - Recognized independent certifying agency for the implementation and coordination of the program.
  - Cooperation between herd owners and between herd owners and dairy/beef industry.
  - Financial incentive for participation in certification program.

- Conditions under which this will not be effective:
  - Poor veterinary services infrastructure, including laboratory capacity.
  - Lack of strong support from the animal industry.

PT1

- Description:
  - Manure storage systems.
  - Manure processing, e.g., composting (not 100%).
  - Manure disposal; season and land use, e.g., forage crops.
  - Biosecurity.

- Advantages:
  - Possible control of multiple fecal-oral pathogens among animals on the farm.
  - Limits environmental contamination and spread to wildlife or humans.

- Disadvantages:
  - May require major change to normal farming practices.
  - Added cost to farming business.
  - Unproven (only theoretical) impact on paratuberculosis control.

- Evidence:
  - Only empirical (MaP/digesters).

- Conditions for effectiveness:
  - Maintenance of system.
  - Compartment.

- Official control or monitoring.

PT2

- Principles:
  - Young animals are most susceptible to MaP.
  - pTB is transmitted fecal-orally.

- Description:
  - Clean calving area.
  - Prompt calf removal from calving area (pen, paddock or pasture).
  - Clean colostrum (from one – preferably test-negative – cow to one calf).
  - Feeding clean (pasteurized) milk up to weaning.
  - Clean calf rearing environment (no contact with manure from adult cows).

- Advantages:
  - Controls multiple fecal-oral pathogens (e.g. Salmonella).
  - Equates with “Good Farming Practices”.
  - Accepted to work by JD experts.
  - Common sense: an important point of intervention.

- Disadvantages:
  - Added cost to farm business (hard to measure, but significant).
  - Benefits are not well documented.
  - May require change in facilities.
  - Easier to implement on large than on small dairies.
  - Not all practices can be applied in beef herds (see below).

- Evidence;
  - Limited data for PT2 alone (i.e., not together with DR1).

Beef cattle management issues:

- Farmers normally do not remove calves immediately, but they can remove the cow-calf pair to a new pasture.
- Farmers may use ‘community’ pastures; commingling of herds.
- Calving pasture.
- Housing /facilities.
- Lower apparent MaP infection prevalence; few farms require control programs, although some have financial problems because of a high incidence of clinical JD (e.g., imported limousine breeds in Scotland).
- Clinical signs of JD may not be obvious.
- Economic impact is less significant relative to dairy farming.
- Implementation of testing in cow-calf operation is limited due to the nature of management.

Conditions under which this will not be effective:

Diagnostic testing without a defined protocol for handling test-positive cattle, e.g. culling or segregation.
Lack of facilities.
Lack of a market for test-positive animals.
Failure of herd owners to perceive economic benefit.
Failure of cattle industries to promote the program.

DR2

- Description:
  - Remove offspring of test-positive (presumably infected) animals.
  - Identify young animals born to test-positive (presumably infected) dams.

- Advantages:
  - Preempts need for other controls later in the life of the animal.
  - Reduces calf-calf transmission.
  - Culls potentially infected individuals.
  - Smaller economic consequences.

- Disadvantages:
  - Potentially high cost / benefit ratio.
  - Limited data on impact of this intervention on overall pTB control.

- Research needs:
  - Cell mediated Immunity (CMI) test for infection identification in cattle <1 year old
  - Data indicate interferon-γ (IFNγ) assay fails in cattle <12 –16 months old.
  - Relative contribution of infection *in utero.*
  - If MaP transmission from dam to calf is at a 1:1 ratio, is then testing the dam sufficient? Will keeping calves separate from test positive dams help?
  - Do all infected calves develop clinical JD at a later age?

PT3

- Description:
  - Any method to prevent adult-to-adult MaP transmission by the fecal-oral route; essentially manure management / hygiene, e.g. not feeding paunch content.

- Advantages:
  - Controls other fecal-oral pathogens of adult cattle.

- Disadvantages:
  - Costly. May require facilities redesign.
  - Limited or no data on the impact of this control strategy on the spread of pTB.

- Research needs:
  - Infectious dose of MaP for adult cattle.

DR4

- Description:
  - Testing cow pats to determine herd MaP infection status.
  - Isolating contaminated natural water sources.
  - Frequent cleaning of water troughs.
  - Controlling contact of cattle with wildlife.
  - Restricting manure movement between farms.
  - Management according to contamination.

- Advantages:
  - Controls multiple cattle pathogens.
  - Low-cost way to identify MaP-infected herds.

- Disadvantages:
  - Additional cost to farm business.
  - Unproven value.
  - Negative test results give limited confidence in MaP-free herd status.

- Evidence:
  - Indirect/theoretical only.
  - Environmental survival data, i.e. data indicate the organism persists a long time in the environment, but it is not known if this represents a true MaP infection risk to animals.

PT4 (+ PT5)

- Description:
  - Wildlife management / restricted entry of other animals.
  - Housing / manure management regarding adult – calf, e.g.,
    - Clean feeding equipment,
    - Clean water supplies,
    - Human movement from young to older animals,
    - Human hygiene: boots and overalls.
  - (Waste) milk management

- Advantages:
  - Controls multiple pathogens.
  - “Good Farming Practices”.
  - Common sense / easily explained.

- Disadvantages:
  - Hard to quantify benefits.
  - Added cost to business, e.g. dedicated feeding equipment.
  - Labor intensive.

- Evidence:
  - Theoretical only.

2.2 PRODUCTS OF PLAN

- Healthier and happier cows.
- More profitable dairy farm.
- Cleaner raw products: milk and meat with lower levels of MaP, easier for processors to manage (destroy residual contamination).
- Confident and contented consumers.

2.3 INTERVENTION PRIORITIES

MaP assumed NOT zoonotic	MaP assumed or perceived zoonotic
PT2 (herd hygiene)	DR1 (using most sensitive test)
DR1 (remove major sources)	PT2 (herd hygiene)
DR0 (control new introductions)	DR0 (close herd)
	PT1 (manure management to limit environmental contamination)
	DR2 (if a test is available)

2.4 KNOWLEDGE GAPS

Dairy

- Is MaP zoonotic?
- What is the infectious dose for cattle by age?
- Can science develop a more effective and marked vaccine?
- What can safely be done with milk and meat from infected cows? (e.g., UHT processing instead of cheese production)?
- Can we safely use feed for calves based on milk and/or whey powder?
- How can we certify feed for freedom of MaP?
- How to inactivate MaP on the farm? Lime treatment of manure/soil?
- How to design facilities to optimise hygiene?
- What is the natural history of the disease?
- When and how did the infection initiate in terms of the production cycle of cattle?
- Can an accurate diagnostic test be developed for young animals?
- Is chemoprophylaxis possible in calves?
- What is the role of wildlife in the ecology of MaP on farms?

Beef (prioritized)

- Young animal test.
- Increased sensitivity and specificity of tests at reasonable/reduced cost.
- Fecal shedding dynamics (timing and quantity).
- Cost-effective management practices.
- Validated environmental sampling in beef herds.
- Interaction between other species, strains, etc.
- Biotechnical process to inactivate MaP.
- Risk of transmission from artificial insemination.
- Adult to adult transmission under different management scenarios (function of density, time).
- Importance of water to the propagation of MaP.

3 Animal product level

3.1 POTENTIAL DISEASE CONTROL POINTS

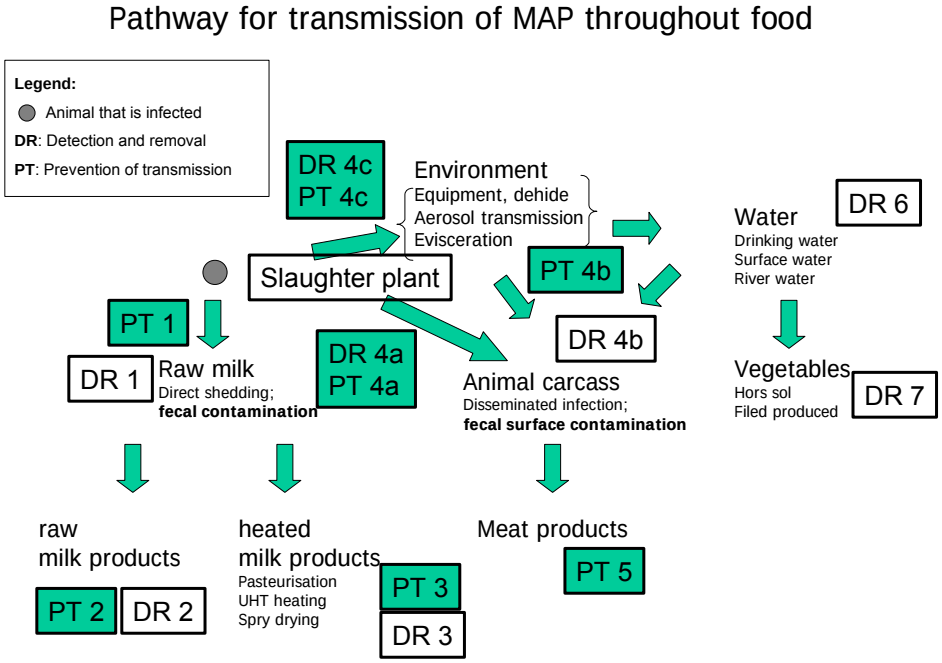

Exhibit 2: Disease control points on animal product level

3.2 MILK

DR1

- Description:
  - Bactofugation/ microfiltration/ ultrafiltration.

- Research need:
  - Effectiveness of commercial bactofugation/ microfiltration/ ultrafiltration processes for removal of MaP.

PT1

- Description:
  - Prevent milk from clinically ill cows from getting into supply chain based on clinical disease signs.
  - Prevent contamination by milking practices, proper udder cleaning with agreed procedures.
  - Proper cleaning of milking equipment.

- Research needs:
  - Studies into effective measures for cleaning milking equipment.

- Ideally, in-line measurement of amount of faecal contamination of milk.
- More knowledge about the dynamics of faecal shedding, particularly quantitative data (cow-level and herd-level).
- Determine quantitative load of MaP in raw milk.
- What is an acceptable number of live/dead MaP per ml of raw milk (for consumption and for processing)?

Sample calculation :
- Herd of 20 cows.
- 1 high shedder, 10^6 cfu/l in feces.
- 10^2 cfu/l directly shed into milk.
- Assume 0.5 g faeces per litre of milk.
- Final concentration of MaP in this cow's milk is estimated at $5x10^5$ cfu/l.
- In farm bulk tank this concentration will be 20 times diluted, $2.5x10^4$ cfu/l.
- Decimal reduction in MaP achieved by HTST pasteurisation at minimum 72 °C for 15 s reported in published studies varies:
  >4 log (72.5 °C, 27 s), Lynch et al., 2007
  >4.2 - >7.1 log, Rademaker et al., 2007
  >4 - > 6 log, McDonald et al., 2005
  4 - 5.2 log, Grant et al., 2005
  5 – 7.7 log, Stabel et al., 2004
  3-6 log, Hammer et al., 2002
  5-6 log, Gao et al., 2002
  >4 - >7 log, Pearce et al., 2001.

If the minimum reduction in MaP achievable by HTST pasteurisation is 3-4 log, then complete inactivation of the above level of MaP in milk from an infected herd should, in theory, be achieved by HTST pasteurisation.

Issues in relation to above calculation:
- Is the above calculation realistic?
- Are the number of CFUs estimated to be present in faeces of the high shedder realistic?
- What is the true impact of pasteurisation on MaP?
- How do we explain low levels of surviving MaP in commercially pasteurised milk?

Critical points:
- Control of contamination by hygienic milking practices.
- Quantity of MaP in the farm environment (faeces) and supply chain.

DR3

- Detection and removal of MaP from processed milk is not feasible.

PT3

- Recontamination is not an issue, no measures necessary for preventing it other then generic quality assurance.

- Research needs:
  - Dynamics of growth of residual viable MaP in milk after processing.

- Is there a problem in UHT milk? What is the clinical relevance?

3.3 PROCESSED DAIRY PRODUCTS

Approach taken by group considering processed dairy products

When designing food manufacturing processes the initial level of the pathogen of concern in the raw materials (H_0) and how the various processing steps impact this initial level, whether they lead to a reduction (ΣR) or increase (ΣI) in numbers of the pathogen present, should be considered. This results in determination of the Performance Objective (PO), the maximum frequency and/or concentration of a (microbial) hazard in a food at a specified step in the food chain before time of consumption (Gorris, 2004; International Commission on Microbiological Specifications for Foods (ICMSF) 2006), which can be derived using the following formula:

$H_0 - \Sigma R + \Sigma I \leq PO$.

For each of the dairy products considered an attempt was made to identify which processing steps should, in theory, lead to reduction in levels of MaP and which could lead to an increase in, or concentration of, MaP. Where relevant information was not available to allow us to reach a conclusion about the impact of a processing step then this area was identified as a research need.

Dairy products considered:

- Cheeses - raw and pasteurised; manufactured using milk from various species; soft, hard, semi-hard
- Fermented products - yoghurt, kefir, butter
- Powdered dairy products

Dairy products not considered: condensed milks, shelf stable dairy

Cheese

Step	Details	Effect
Raw milk	Variable level of MaP	Starting level
Reduction treatment	Pasteurisation Bactofugation Thermisation	Inactivation Removal Inactivation
Starter	pH decline	Minimal effect at this step
Curd formation	Separation of whey	Concentration x 10; some loss with whey
Pressing	Complete removal of whey	Minimal effect
Ripening	pH, a_w, salt	Slow reduction over time
Packaging and storage	Chilled storage	No effect, except due to ripening

- Knowledge gaps:
  - Distribution of MaP upon separation of curds and whey.
  - Effect of salt concentration on MaP survival.

- Effect of reduction treatments (bactofugation, thermisation) or combination treatments.

Yoghurt

Step	Details	Effect
Raw milk / thermized	Variable level of MaP	Starting level
Milk powder	Initial level of MaP	Increase of MaP?
Reduction treatment	Pasteurisation 90 to 92 °C	Inactivation
Starter	pH decline to 4.5 or below within 12 – 18h	Minimal effect at this step?
Addition of fruit, nuts or other inclusions	Varying heat treatments could affect MaP levels	Introduction of low, sporadic contamination?
Distribution and storage	Chilled shelf life ≤ 30d	Decrease of MaP?

- Knowledge gaps:
  - Survival of MaP under fermentation and storage conditions.
  - Risk of MaP contamination of yoghurt via added ingredients.
  - Effectiveness of heat treatment at 90-92°C.

Butter

Step	Details	Effect
Raw milk	Variable levels of MaP	Starting level
Separation of cream	Partitioning of MaP	Some loss in whey ?
Reduction treatment	Pasteurisation 95 to 110 °C for a few seconds	Some reduction of MaP
Starter	pH decline to 5.5 in a few hours	No effect
Churning / salting	Separation of butter milk	Some loss in butter milk
Washing		Loss or potential recontamination from water
Storage	Chilled or frozen storage, long shelf life	Minimal reduction?

- Knowledge gaps:
  - Effect of pasteurisation process applied to cream (vs whole milk; vs standard pasteurisation conditions).
  - Segregation of MaP in fat and skim.
  - Segregation of MaP upon separation of buttermilk.

Powdered milk products

Step	Details	Effect
Raw milk	Variable level of MaP	Starting level
Separation of cream	Partitioning of MaP	Movement with skim milk or cream ?
Reduction treatment	72°C – 15s	Reduction of MaP
Spray drying	Various conditions used; est highest particle temp. 70°C	Concentration with evaporation; some reduction due to heat
Rotary dryer	Higher temperature 120°C	More reduction than spray drying alone ?
Cooling / filling	Filling at ambient conditions	Prevent recontamination
Storage and distribution	Dry conditions	No effect

- Knowledge gaps:
  - Is double heat treatment an option to ensure MaP inactivation/elimination?
  - What is the survival / inactivation of MaP in dried milk products?
  - For products using powdered milk, such as infant milks – what is the contribution of MaP from other ingredients?

Role of testing for dairy products

- Raw materials
  - To verify levels of MaP in raw materials received, particularly when expected levels are close to reductions achievable during processing.
- Hygiene indicators
  - To assess raw material hygiene status.
  - To evaluate cleaning and sanitation of process environment.
  - To verify effectiveness of reduction steps.
  - As an indicator of recontamination events.

Common processing steps and their contribution to PO:

- MaP in raw milk and other raw materials (H_0)
- Heat treatment (R)
- Fermentation / acidification (R?)
- Concentration (coagulation; spray drying) (I)
- Salt level (R?)
- Ripening period / survival during shelf life (R)
- MaP in inclusions / components added after heat treatment (I?)
- Good manufacturing practices, zoning preventing recontamination (controlling I)

General knowledge gaps

- What should the Performance Objective (P.O.) be in relation to MaP in dairy products?
- What is an acceptable level of MaP in the finished product?

- Should we be concerned about dead MaP cells?
- Who is the population at risk?
- Effect of disinfection procedures in MaP.
- Cross-contamination risk of MaP on processing equipment and in processing environment.
- MaP in process water (incl. recycled).
- Resolution of difficulties in interpreting pasteurisation data.

3.4 BEEF

Detection

- Prior Knowledge- History of the herd of origin.
- Segregation - high vs. low risk.
- Anti-mortem testing.
- Body condition score – emaciation.

Removal (if MaP is zoonotic)

- Exclusion of carcass/animals.
- Partial removal of high risk organs/tissue.
- Treatment - product disposition.

Advantages

- Avoid contamination – no further specific action is needed.

Disadvantages

- Sensitivity / specificity issues.
- Time.
- Cost.

Research needs

- Potential growth and its rate of MaP in meat products.
- Relationship between visual inspection and MaP infection for both ante-mortem and post-mortem).
- Ante-mortem: BCS and other ante-mortem predictors.
- Post-mortem: changes in gross pathology.
- Determination of the effectiveness of current fecal contamination (surface contamination) mitigation measures on MaP.
- Determination of which tissues are more likely to transmit MaP infection.
- Identification of processing systems that will properly inactivate MaP infectivity (e.g. thermal processing of exposed carcasses/raw materials).
- Determination of effectiveness of natural pH reduction of aging meat in MaP viability.
- Effectiveness of routine cooking procedures in deactivation of MaP (temperature).
- Microwave, grill-top, baking/roasting.

- Analysis of ground meat products for disseminated MaP – determination of presence and levels.
- Development of rapid screening tests for product verification testing.
- Effectiveness of standard rendering techniques for MaP inactivation.
- Importance to know if dogs and cats susceptible to MaP infection (in the event that MaP positive carcasses are rendered into meat and bone meal).

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