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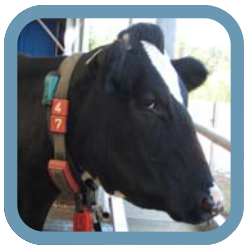

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Ministry of Agriculture, Food and Rural Affairs

Ontario

Estimating *Mycobacterium avium paratuberculosis* Prevalence from Laboratory Testing

Ann Godkin and Kathy Zurbrigg, Veterinary Science and Policy Unit, OMAFRA, and Bev McEwen, Animal Health Laboratory, University of Guelph

Many herd owners and their veterinarians have increased their testing of cattle for Johne's Disease. They may be testing individual cattle to confirm clinical signs as being caused by *Mycobacterium avium paratuberculosis* (MAP) infection, or whole herds to estimate the extent of infection within a particular herd.

One way to estimate the extent of MAP infection in Ontario dairy herds would be to summarize all laboratory testing results.

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Testing for MAP in Ontario is done predominantly in two laboratories. Most tests of herds or large numbers of cows are done at CanWest DHI in Guelph, using the milk ELISA test on test-day milk samples. Testing of individual cows with clinical signs suspected to be Johne's disease, or for other reasons, is done primarily by submission of samples to the Animal Health Laboratory (AHL) in Guelph, Ontario. The AHL tests serum using ELISA or manure by culture or direct fecal PCR.

Ideally, results from both laboratories would be combined to give the overall picture of MAP in Ontario. Unfortunately, herds do not currently have a common identifier used by both laboratories that would enable us to link results; thus, we can only provide summaries from each laboratory separately.

In **Ceptor** October 2011, we provided a summary of MAP test results from herds enrolled in the Ontario Johne's program. Updates are provided periodically on the program website at *www.johnes.ca*. This information predominantly reflects testing done at the CanWest DHI milk-testing laboratory. To date, just over 50% of eligible herds have participated in the program. Among the participants so far, about 23% of herds have had a test-positive animal and 0.7% of all cows tested have been test positive.

For testing of individual or small groups of cows, results come from the AHL. From May 2007 until the end of November 2011, there were 3,081 submissions for MAP testing by fecal culture, direct fecal PCR or serum ELISA. A submission could include single or multiple samples and single or multiple test types. A submission was considered positive if one or more of the samples in that submission tested positive by any test.

(Continued on page 3)

Figure 1 illustrates the numbers of samples submitted and the proportion positive by year. During this time period, 1,231 (40%) of submissions had at least one positive result. Of the 18,593 individual samples tested, 1,662 (9%) were positive. Unfortunately, the owner information provided was frequently incomplete and we could not determine when multiple submissions came from the same farm or repeatedly from the same animals.

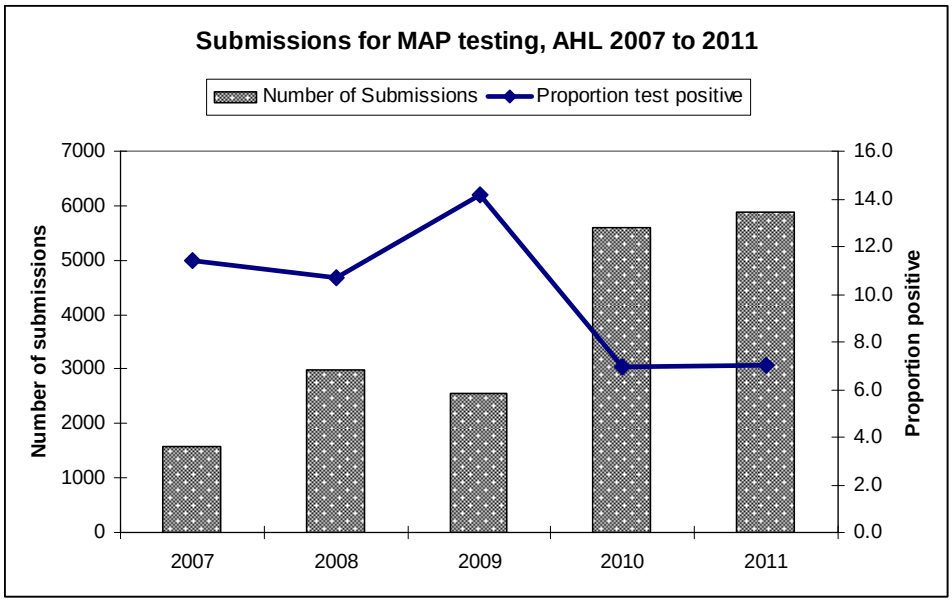

Figure 1

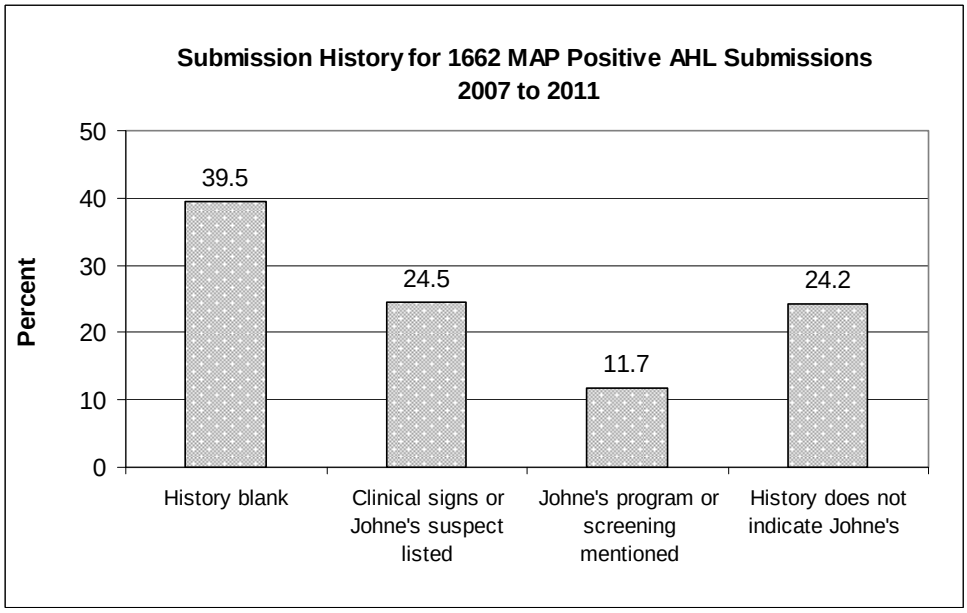

Figure 2

We were interested in looking at the reasons owners and veterinarians submitted samples to the AHL. To investigate this, we summarized the history information provided on the AHL submission form. The investigation was limited to the 1,662 positive samples. The text was searched manually and the reasons were grouped into four categories: no history (blank), a suspicion of Johne's causing clinical signs, as part of a herd screening program (i.e. dry cows) or because clinical signs were present but Johne's was not mentioned as being suspected.

(Continued on page 4)

Figure 2 displays the breakdown of the reasons given for testing.

In the last five years the number of submissions for MAP testing at the AHL has increased. A higher proportion of samples have been test-positive at the AHL than at CanWest DHI (10.0% vs 0.7%). Although the number of samples tested at AHL has increased each year, the proportion positive has remained about the same until 2010 and 2011, when the proportion positive declined.

Reasons for testing of cattle that ultimately were test–positive were not provided on about 40% of the submission sheets. Of the test-positive animals where a reason was provided, it appears that about 40% have a history of signs of clinical Johne's disease, 20% were tested as part of Johne's screening and another 40% had no mention of Johne's disease at all.

Work is underway at the Ontario Veterinary College to develop a link between the two laboratories so that a better description can be developed. Submitting veterinarians are strongly encouraged to provide a history with all submissions to the AHL. In particular, for situations where MAP testing is done, we would like to be able to determine how often cows suspected of having Johne's disease do turn out to be test positive.

Testing for Vitamin D_3 in Ontario Swine Herds

Paisley Canning, Veterinary Student, Ontario Veterinary College, University of Guelph, and Tim Blackwell , Veterinary Science and Policy Unit, OMAFRA

Vitamin D_3 is an essential nutritional component of swine diets. It regulates calcium and phosphorus absorption and metabolism, and appears to regulate other metabolic processes in animal cells. Pigs naturally produce vitamin D_3 after exposure to sunlight, but swine rations are supplemented with vitamin D_3 because most conventional swine rearing facilities prevent pigs from exposure to direct sunlight. Deficiencies in vitamin D_3 negatively affect pig growth and have been associated with the "humped back" skeletal deformity occasionally reported in growing swine.

Vitamin D deficiency is not commonly diagnosed in Ontario. If you suspect a vitamin D deficiency, two metabolites of vitamin D_2 and D_3 called 25-hydroxyvitamin D (25(OH) D) can be measured in serum. These metabolites are used to measure the amount of vitamin D that was consumed, absorbed and available to the pig. Recent reports from Kansas associate low serum 25(OH) D concentrations (< 15 ng/mL) in suckling pigs with increased piglet mortality in the farrowing house and nursery [1,2] . A reliable and effective means of testing for 25(OH) D levels in feed and sera is important to diagnose or rule out this possible risk factor for piglet mortality in Ontario.

A pilot project was undertaken to compare vitamin D_3 concentrations in swine feed on four Ontario swine farms. The pigs on these four farms did not have clinical signs consistent with a vitamin D_3 deficiency. Feed samples were sent to two different diagnostic laboratories for testing and comparison purposes.

Results from feed analyses for Vitamin D performed at both laboratories were dramatically different from the concentrations indicated by the feed manufacturer. Results from testing of split samples indicated a low level of repeatability within each laboratory. The amount of 25(OH) D_3 in sera is the parameter of most concern to swine practitioners and producers; therefore sera, rather than feed, was identified as the preferred sample for testing to determine if adequate levels of vitamin D_3 concentrations were being supplied.

Sera were collected from growing pigs between 8 and 25 kg on two of the four farms where rations had been assayed for feed vitamin D_3. The serum samples were split and sent to the same two diagnostic laboratories [a,b] for 25(OH) D_3 testing that were used for the feed analyses. Results for 25 (OH) D_3 concentrations in sera are reported in either ng/mL or nmol/L. To convert ng/mL to nmol/L the value in ng/mL is multiplied by 2.4940. For example, 15 ng/mL, the lower limit of the normal range for suckling pigs, is equal to 37.41 nmol/L.

(Continued on page 5)

The normal range reported by Drs. Ron Horst and Jesse Goff at Iowa State University for nursery and finisher pigs is 25 to 35ng/mL, equal to 62 to 87 nmol/L. (1)

The results for Farm A are shown in **Figure 1** and the results for Farm B are shown in **Figure 2**. Both laboratories reported similar concentrations on the split serum samples. According to the recommendations from Iowa State, both farms appear to have lower than "normal" 25(OH) D concentrations in the sera of the pigs sampled.

Further work is planned to determine the serum values for 25(OH) D that are associated with maximum growth and productivity in the sera of growing swine in Ontario.

a. Total 25(OH)D_3 by Liaison® at Heartland Assays, LLC. 2711 South Loop Drive, Suit 4400, Ames, Iowa 50010.
b. Order code: 20001 Serum 25-Hydroxyvitamin D. Diagnostic Center for Population and Animal Health, 4125 Beaumount Road, Michigan State University, East Lansing, Michigan 48910-48104.

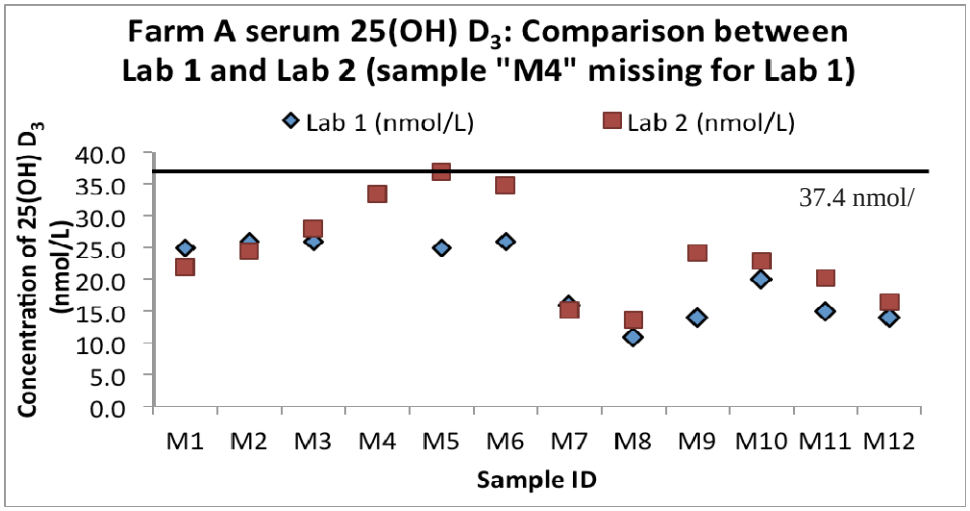

Figure 1. Serum 25(OH) D_3 concentrations in the sera of growing pigs (8 to 25 kg) on Farm A. Samples M1 to M6 are from pigs 8 to 14 kg and M7 to M12 are from pigs between 15 and 25 kg of weight. The line drawn at 37.4 nmol/L is the lower cut-off value for newly weaned pigs (< 8 kg). Older pigs would be expected to have approximately twice this concentration of vitamin D_3 in their sera.

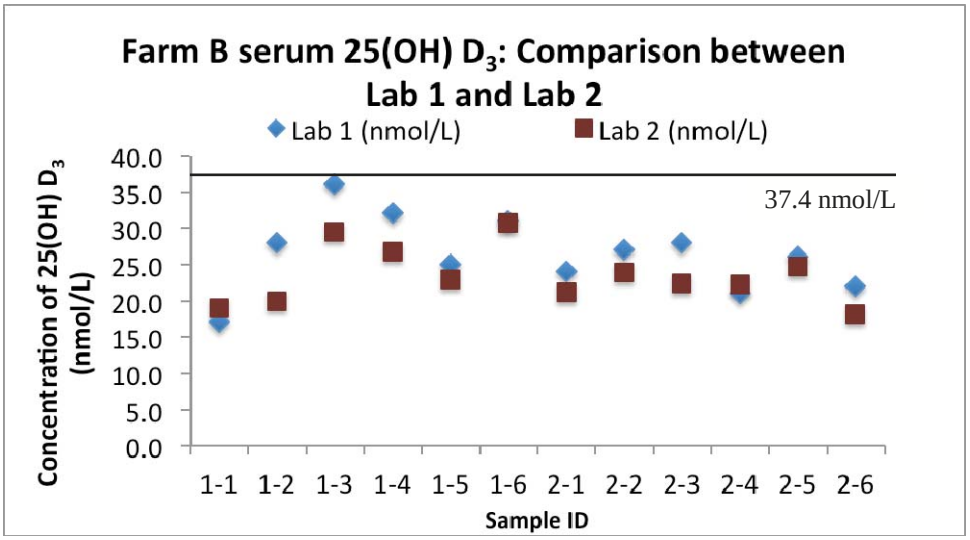

Figure 2. Serum 25(OH) D_3 concentrations in the sera of growing pigs (8 to 25 kg) on Farm B. Samples 1-1 to 1-6 are from pigs between 8 and 14 kg of weight and samples 2-1 to 2-6 are from pigs between 15 and 25 kg of weight. The line drawn at 37.4 nmol/L is the lower cut-off value for newly weaned pigs (<8 kg). Older pigs would be expected to have approximately twice this concentration of vitamin D_3 in their sera.

(Continued on page 6)

1. *Henry S, Tokach L, Potter M. Failure to Thrive—What's all this about Vitamin D. K-State Swine Day, 2011.*

2. *Flohr JR, et al. The Effects of Orally Supplemented Vitamin D_3 on Serum 25 (OH) D_3 Concentrations and Growth of Pre-Weaning and Nursery Pigs. In Swine Day 2011; Report of Progress 1056. pp. 34-45. Kansas State University, Agricultural Experiment Station and Cooperative Extension Service.*

On-going Problems with the Interpretation of ELISA Results for *Mycoplasma hyopneumoniae* Antibody Determination in Swine Sera

Tim Blackwell and Janet Alsop, Veterinary Science and Policy Unit, OMAFRA

In November 2010, routine testing of a high-health swine-breeding stock herd resulted in a five-week period without sales because of unexpected positive results for *Mycoplasma hyopneumoniae* antibodies. Testing was done using the Oxoid ELISA test kit at the Animal Health Laboratory, University of Guelph. On three consecutive test dates, the results for two of 20, three of 60 and 11 of 30 animals were either suspicious or positive. During the previous five years of testing in this herd, where 20 animals were tested twice yearly, a positive or suspicious test result had never occurred. The positive and suspicious samples from these animals were sent for testing with the *M. hyopneumoniae* IDEXX ELISA at the University of Montreal. All of the positive and suspicious samples tested negative, with the exception of one animal, which had also tested positive with the Oxoid test. This animal and one of the suspicious animals were sent for necropsy at the AHL. All lung samples were negative for *M. hyopneumoniae* by polymerase chain reaction testing and the herd has remained free of clinical signs of *M. hyopneumoniae* infection during the last 12 months. On two subsequent test dates, each of 20 market-age swine tested from the herd, have been negative for *M. hyopneumoniae* antibodies using the Oxoid test.

In November 2011, on routine testing of an unrelated herd, which also contained high-health swine-breeding stock, seven of 24 samples tested either positive or suspicious for *M. hyopneumoniae* using the Oxoid ELISA test. There were no clinical signs of *M. hyopneumoniae* infection in the herd. These seven samples were tested at the University of Montreal using the IDEXX ELISA test and all seven tested negative. The Animal Health Laboratory at the University of Guelph re-tested the seven samples using the Oxoid test and found the results demonstrated poor repeatability, with a distinct trend for samples to test negative on repeated sampling.

Although the Oxoid ELISA has been reported to have a slightly higher sensitivity and specificity than the IDEXX ELISA, these two recent events should alert practitioners to be cautious in their interpretation of unexpected positive or suspicious s/p ratios when using the Oxoid ELISA to determine *M. hyopneumoniae* antibodies in swine.

The Use of CIDRs in Small Ruminants – Revised Instructions

Jocelyn Jansen, Veterinary Science and Policy Unit, OMAFRA and
Chris Buschbeck, Markdale Veterinary Services, Markdale, Ontario

Controlled Internal Drug Releasing (CIDR) devices are an intravaginal insert containing the naturally occurring hormone progesterone. They can be used "in season" to synchronize estrus in a group of ewes, "out of season" to bring ewes into heat when they are not naturally cycling and/or in artificial insemination programs.

CIDR devices (CIDR© 330, Pfizer) became available on the Canadian market for sheep in early 2011. It is the first ovine product approved/licensed through the Minor Use and Minor Species initiative with the Veterinary Drugs Directorate (Health Canada). This program streamlines the drug approval process, allowing information about a drug, its effectiveness and safety, to be used in Canada based on data from another country. The goal is to increase the number of animal health products available in Canada for the minor species (sheep, goats, deer, elk, wildlife, rabbits, caged birds, etc).

The Canadian CIDR label was based on independent research performed in the United States [1,2] and is as follows:

- indicated for the induction of estrus in ewes during seasonal anestrus
- insert CIDR for 5 days
- no PMSG at time of CIDR removal
- use a ram to ewe ratio of 1:18
- not for use in ewes that have never lambed

Unfortunately, the conditions described in the research do not reflect the Canadian "in season" or "out of season" situation. The result will be low pregnancy rates (0 - 30%) when the label directions are followed. To combat this, these recommendations should be followed to ensure a higher pregnancy rate (≥ 60%, rates will depend on breed and time of year).

- insert CIDR for longer than 5 days (see Tables 1-4)
- if using CIDRs out of season, PMSG must also be administered
- use a ram to ewe ratio of 1:5-6 out of season or 1:10 in season
- use of CIDRs in maiden ewes will work but they must be sexually mature (65-70% of their adult weight and have had at least one natural heat cycle)

Tables 1 and **2** provide specific information on "in season" programs, while **Tables 3** and **4** provide information on "out of season" programs.

Additional Information

- These recommendations constitute extra-label drug use, which necessitates a veterinary prescription and a valid veterinary-client-patient relationship.
- Ewes must not be slaughtered for 24 hours after CIDR removal. There is no milk withdrawal required.
- CIDRs are not labelled for use in goats (extra-label drug use). CIDRs should be inserted for 17 days in goats and the buck introduced 18-24 hours later. Goats can also be short cycled with prostaglandin on day 9 following the schedules in Tables 2 or 4.
- Remind producers to be as clean as possible when inserting the CIDRs to avoid vaginal irritation.

Table 1. CIDR Schedule for Sheep – In Season

Day	Protocol
1	Insert CIDR
14	Remove CIDR
15	Introduce ram 18-24 hours after CIDR removal

Dr. Chris Buschbeck, Proceedings SRVO Spring Meeting, March 3, 2011.

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Table 2. CIDR Schedule for Sheep – In Season (optional short cycle)

Day	Protocol
1	Insert CIDR
9	Inject prostaglandin (0.75 ml Estrumate® or 2 ml Lutalyse®)
11	Remove CIDR
12	Introduce ram 18-24 hours after CIDR removal

Dr. Chris Buschbeck, Proceedings SRVO Spring Meeting, March 3, 2011.

Table 3. CIDR Schedule for Sheep – Out of Season

Day	Protocol
1	Insert CIDR
14	Remove CIDR AND inject PMSG (500 IU Pregnecol®, Folligon® or Novormon™)
15	Introduce ram 18-24 hours after CIDR removal

Dr. Chris Buschbeck, Proceedings SRVO Spring Meeting, March 3, 2011.

Table 4. CIDR Schedule for Sheep – Out of Season (optional short cycle)

Day	Protocol
1	Insert CIDR
9	Inject prostaglandin (0.75 ml Estrumate® or 2 ml Lutalyse®)
11	Remove CIDR AND inject PMSG (500 IU Pregnecol®, Folligon® or Novormon™)
12	Introduce ram 18-24 hours after CIDR removal

Dr. Chris Buschbeck, Proceedings SRVO Spring Meeting, March 3, 2011.

1. *Knights M, et al. Short-term treatment with a controlled internal drug releasing (CIDR) devise and FSH to induce fertile estrus and increase prolificacy in anestrous ewes. Theriogenology 2001;55:1181-1191.*

2. *Knights M, et al. Effectiveness of intravaginal progesterone inserts and FSH for inducing synchronized estrus and increasing lambing rate in anestrous ewes. J. Anim. Sci. 2001;79:1120-1131.*

Brisket Locator Basics

Neil Anderson, Veterinary Science and Policy Unit, OMAFRA

Brisket locators make stalls less comfortable for cows. Cows prefer to use stalls without brisket locators and spend less time lying in stalls with brisket locators. However, brisket locators may contribute to cleaner beds and cows, and reduced risk of mastitis. The challenge is to design, select and install a brisket locator that meets the needs of the cow for comfort and the owner for stall cleanliness. Here are several design principles and features that may be useful when judging, selecting or installing a cow-friendly brisket locator in a free-stall barn.

Design Principles

1. Permit normal behaviour
   a. Stride when rising
   b. Legs extended when resting
   c. Hours of resting
2. Assure a safe resting place
   a. Free of hazards (e.g., entrapment, dirty bed)
3. Enhance stall cleanliness
   a. Resting parallel to the loops
   b. Resting close to the alley curb (forward location)

(Continued on page 9)

4. Be practical
    a. Strong enough to provide long service (durable)
    b. Easy to install
    c. Reasonably priced

Design, Construction, and Installation Details and Justifications

1. Height & width
    a. ≤4 inches above the mat or mattress.
    b. A stride (step forward by one leg) is part of a normal rising motion.
        i. Brisket locators higher and wider than 4 inches (for mature Holsteins) interfere with the stride.
        ii. Brisket locators higher and wider than 4 inches may contribute to cows stumbling or falling forward.
    c. Resting with one or both forelegs extended forward is a normal resting posture.
        i. Brisket locators higher than 4 inches interfere with this behaviour.
        ii. Cows rest diagonally in the stall when high brisket boards interfere with normal resting postures.
    d. Unwanted outcomes from oversized brisket locators include diagonal resting, manure and urine on the beds, pressure sores on spines, reduced hours of resting, or restlessness and hock sores.
2. Mount
    a. Attach to the platform, not to the loop.
    b. Allows cows to stretch their forelegs under the loop.
    c. Eliminates a hazard for leg entrapment or injury.
3. Space between brisket locator and bottom pipe of the loop
    a. No less than 5 inches
    b. Allows cows to assume the legs forward resting posture
    c. Assures a leg or foot will not be trapped or injured
4. Forward location
    a. For Holsteins, 68, 70 or 72 inches
    b. Measured from the alley curb to the cow-side face of the locator
    c. Defines the forward position of a resting cow
    d. Allows all body parts (including the tail) to rest on the bed
    e. Provides the bed length needed to rest parallel to the loops
5. Shape
    a. Surface should be contoured and smooth
    b. Provides comfort or safety when cows extend and retract their legs
6. Area in front of the locator
    a. Height similar to the stall platform
    b. Provides a safe and comfortable surface for the stride when rising
    c. Provides a comfortable space for legs when extended while resting
    d. Provides excellent traction (not slippery)
    e. Assures ease of rising
    f. Minimizes risks of stumbling or falling
7. Heifer stalls
    a. Design principles are similar to those for cows
    b. Shape and installation features are similar to those for cows
    c. Choose height and width appropriate for the age of heifers in the pen
    d. Provide sufficient space between the top of the locator and bottom pipe of the loop to prevent entrapment of legs
        i. 2 to 2.5 inches for 6 to 12-month-old heifers
        ii. 3 to 3.5 inches for 13 to 20-month-old heifers

The design principles and features listed above provide guidelines for judging the merit of wooden boards, concrete or wood or rubber or plastic curbs, nylon straps, or metal or poly pipes, as safe and comfortable brisket locators. Some may be good choices while others provide too many hazards and challenges to merit consideration for use in our Ontario free-stall barns.

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Figure 1. The plastic curb shown in these photos is a ten-year-old design that allows cows to rest with their front legs extended forward. To prevent entrapment associated with brackets attached to the loop, the locator mounts to the floor; there is a 5-inch space between the top of the brisket locator and the bottom pipe of the loop. The low height allows cows to take the normal stride when rising and the area forward of the curb provides a surface with good traction.

Figure 2. The 6-inch PVC sewer pipe shown in this photo is an innovation in a barn built in 2011. It attaches to the bottom pipe of a loop with metal bracket and provides a 3-inch space for trapping a front leg. It also is too high and too wide for a normal stride when rising and it is an impediment to the legs forward resting posture. It contributed directly or indirectly to injuries to cows in this barn.

(Continued on page 11)

Figure 3. This photo shows a more cow-friendly installation of round plastic pipe as a brisket locator. Floor mounts and a 5-inch space between this black plastic pipe and the loop allow cows to extend their legs forward under the loop without the risk of injury or entrapment.

Results from a Project to Examine Risk Factors for Prototheca Mastitis in Ontario Dairy Herds

Laura Pieper and David Kelton, Department of Population Medicine, University of Guelph, and Ann Godkin, Veterinary Science and Policy Unit, OMAFRA

Prototheca is an alga that can cause mastitis in dairy cows. It is widespread in the environment and grows well in moist places, such as dirt, wet bedding, left-over feed and water. Infected cows usually show mild signs of mastitis with occasional flakes and increased somatic cell counts. Infected cows can shed the algae intermittently, with periods without positive culture results lasting for months. Unfortunately, there is no treatment for Prototheca mastitis. Herds appear to be at risk of subsequent infections following initial diagnosis. Over the last ten years, this type of mastitis seems to be emerging in Ontario. Therefore, a study was conducted to investigate farm-level risk factors for this disease.

Twenty-three case farms, identified by their herd veterinarians as having had repeated cases of Prototheca-positive cultures for mastitis within the last two years, were enrolled in the project. For each case herd enrolled, the veterinarian submitted four herd names for selection of a matching control herd. Prospective control herds had to have approximately the same number of milking cows and the same style of cow housing (free-stall or tie-stall) as the case herd, and have had no Prototheca-positive milk cultures over the two-year time period. Each case and control farm was visited once between January 2011 and May 2011. Milk samples were collected from the milking herd and a questionnaire was administered to the herd manager. Farm characteristics, herd management, milking technique and mastitis treatment practices were compared between case and control farms.

Results

The average prevalence of Prototheca-positive milk cultures among the case herds was 5.1% (0.0-2.5%).

(Continued on page 12)

The number of culture-positive cows found was usually higher than the producer predicted. The study indicated that extra-label infusion of injectable drugs into the udder (e.g., Excenel, Predef, or Penicillin), use of more numerous, different injectable and intramammary antibiotics, and administration of a teat sealant (internal or external) were risk factors for being identified as a case farm. It was concluded that inappropriate treatment protocols and poor intramammary infusion hygiene were associated with Prototheca mastitis.

Recommendations for Herd Owners where Prototheca Mastitis is Diagnosed

As a result of the project and using information gathered from additional scientific publications, the following general recommendations can be made to owners of herds where Prototheca mastitis is occurring:

1. Stop treating cows diagnosed with Prototheca mastitis. Prototheca mastitis is incurable.
2. Culture cows with mastitis prior to treatment.
3. Separate/quarantine Prototheca-culture-positive cows away from the herd. Milk infected cows last. Remove these cows from the herd as soon as reasonable.
4. Culture milk samples from cows with mastitis or high SCCs (cows with SCCs greater than 200,000 cells/mL) routinely. Only culture will identify Prototheca-infected cows. Prototheca-infected cows cannot be recognized by any characteristic appearance of signs, the milk or SCC profile.
5. In a herd with a history of Prototheca, periodically test all cows for mastitis.
6. Improve the lactating and dry cow housing and environment to keep teat ends free of contamination from environmental bacteria and alga.
7. Improve milking hygiene – ensure teats are thoroughly cleaned and dried prior to milking.
8. Review treatment protocols, particularly protocols for treating and testing cows whose mastitis is not resolved with first antibiotic treatment per lactation.
9. Train staff that treat cows on proper intramammary infusion technique and teat end hygiene.

Full results from the study will be published in the Canadian Veterinary Journal (accepted).
The project was conducted with funding provided by the Ontario Ministry of Agriculture, Food and Rural Affairs.

Beware—Sulfa Urea Cream Use in Dairy Cows!

Ann Godkin, Veterinary Science and Policy Unit, OMAFRA

Recently, in Manitoba and Ontario, milk inhibitor violations have occurred associated with the use of a topical udder cream on the teats of dairy cows. The product, Sulfa Urea Cream, from Dominion Veterinary Laboratories and P.V.L. (Professional Veterinary Laboratories) has the potential to be sold either over the counter and/or by veterinary practices in different provinces. The product contains two sulfa compounds and when applied to the teats of dairy cattle, sheep or goats before milking can contaminate milk with readily detected sulfa residues.

The product label indicates the product is for use for "the treatment of infections caused by susceptible gram-positive and gram-negative bacteria. Use for the treatment of pyoderma." The label does not indicate the class of livestock or species of animal for which the product is licensed. The label does not indicate if milk or meat withholds are required following use in food-producing animals. Because there are no indications on the label for use in cattle, the use of Sulfa Urea Cream for the treatment of dairy cattle is an extra-label use of this product. In Ontario such use requires the producer to use the product only under veterinary prescription.

In recognition of the risk for residues, in Ontario some practices that sell this product have added a warning sticker to the label to remind producers that sulfas are antimicrobials and that a milk withhold applies if the product is used for the treatment of lactating cows. Other practices have placed the product in a special section behind the counter in the front office that makes it necessary for a producer to request the product specifically and directs clinic staff to have a potential purchaser talk to a veterinarian before dispensing.

Footbath Logistics – Make them work!

Ann Godkin, Veterinary Science and Policy Unit, OMAFRA

Foot baths are commonly used for prevention of Bovine Digital Dermatitis (BDD). They work well provided they are correctly implemented. Investigations of BDD outbreaks often find that the footbath concentration is not at effective levels. This happens because calculations are incorrect, mixing directions are not carefully followed, or the bath is not being refreshed as often as is needed.

All products used in footbaths for treatment of cattle in Canada are an extra-label use (ELUD) as there are no products approved for treatment of BDD. For ELUD for footbaths, a veterinarian with a valid veterinary-client-patient relationship (VCPR) must provide a producer with a prescription that gives directions for product use and enables the producer to use the products in a manner that gives efficacy, avoids residues in meat or milk and provides the cautions needed to ensure the safety of people and animals (target and non-target).

To run an effective footbath, a producer will need to:

1. Have a footbath of adequate size.
2. Calculate the volume of the footbath, allowing for sufficient depth of solution.
3. Mix the chemical or treatment product at the correct concentration.
4. Change the footbath content completely every 150 (dirty feet) to 250 (relatively clean feet) cow passages.

1. Footbath size

***Table 1* gives recommendations for the minimum and maximum sizes for footbaths.**

The Dairyland Initiative group in Wisconsin has provided recommendations for footbath sizing based on scientific assessment of cow behaviour and footbath effectiveness.

Length – The 10-ft. (3.0-m) minimum is established so that, when a cow goes through the footbath, the rear feet of each cow will have at least two steps (hoof immersions) in the bath. When a footbath is too short (i.e. only 6 feet long), only 53% of cows will immerse their rear feet twice in the bath. With a 10-foot bath, 96% of cows immerse both hind feet twice. The 12-ft. (3.7-m) maximum is listed in Table 1 to accommodate the situation where two short (6-ft) trays are used and placed end-to-end to make one larger, more suitable, footbath.

Width – The 24-inch (0.6-m) minimum recommended width is narrower than the width of a typical cow, but easily within the hoof-tracking width. If this minimum is used, sloped boards will be needed as sidewalls to prevent cows missing the bath by stepping sideways. The maximum recommendation of alley width (30 inches, .76 m) accommodates farms where large numbers of cows are leaving the parlour and it is not feasible to slow cow exits with a narrow footbath.

Solution Depth – A 4-inch (9-cm) minimum depth of solution ensures hooves are covered to above the coronary band and dew claws.

Step-in Height – Side walls of the footbath of 10 inches (25 cm) will keep solution from splashing out of the bath. Cows step in and out of a bath of this height without problems.

2. Calculating the volume of the footbath

Table 1 gives examples of footbath volumes at the recommended minimum and maximum sizes. The equation for calculating footbath volume is Length x Width x Height (solution depth). Volume can be calculated in cubic feet if all measurements are in feet, or as cubic meters if all measurements are in meters.

Once the footbath is measured and the volume (cubic feet or cubic meters) is calculated, then the volume units need to be converted to liquid measures as either gallons (US or Canadian/ Imperial) or litres. I recommend that you work in litres.

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Many producers in Ontario have been using recipes for footbath solutions provided by US publications or sources. These US recipes report calculations in US gallons. A US gallon is only about 80% of the size of a Canadian gallon (1 US gallon = 3.79 litres and 1 Cdn. gallon = 4.55 litres). If you have used information from the US, you should adjust the calculations for Canadian sizing. If you use the footbath chemical in the amount recommended for a US-gallon footbath, and add it to a footbath for which you have calculated the volume in Canadian gallons, you may not be adding enough chemical/ treatment.

For example

If you add the amount for a 50-gal footbath from a US recipe, which is calculated for a 50-US-gal bath, when you really have a 50-Cdn-gallon footbath, then you will not be adding enough treatment product. From **Table 2** you can see that a 50-gal US bath needs 6,400 grams (6.4 kgs) of CuS04 for a bath of 4% concentration but a 50-gal Cdn bath is larger and, therefore, needs 6,750 grams (6.75 kg) to make the same 4% concentration.

Table 1. Recommendations for the Minimum and Maximum Sizes for Footbaths

	Footbath Sizing	
Dimensions:	**Minimum Size Footbath** *Feet/Metric*	**Maximum Size Footbath** *Feet/Metric*
Length	10 ft / 3.0 m	12 ft / 3.7 m
Width	2 ft (24 in) / 0.6 m	2.5 ft (30 in) / 0.76 m
Solution Depth	.33 ft (4 in)/ 0.09 m (9 cm)	.33 ft (4 in) / 0.09m (9 cm)
Resulting footbath volume	6.6 cu ft / 0.16 cubic m	9.9 cu ft / 0.25 cubic m
Volume of liquid required	41 Gals (Cdn.) / 160 litres 50 Gals (US)	62 Gals (Cdn.) / 250 litres 74 Gals (US)
Maximum cow passages	150 to 250 cow passages - depending on cow cleanliness (i.e. 200 cow passages is a 50-cow herd for 4 milkings (2 days) or a 100-cow herd for 2 milkings (1 day).	

Useful Constants to Know:

1 cu foot = 7.48 US gallons
1 cu foot = 6.23 Imperial/Canadian gallons
1 cu meter = 1000 litres

1 US gallon = 3.79 litres
1 Imperial/Canadian gallon = 4.55 litres
1 gram =.0022 lbs

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3. Calculating how much chemical/treatment to add
Two examples are provided.

For Copper sulphate, footbath concentrations of 3 to 5% are recommended.

Table 2. Calculations for a Copper Sulphate Footbath

		Calculations for Recommended Footbath Sizes	
Concentration in footbath To make:	Grams of chem./ treatment to add per litre of water in footbath for this concentration	**MINIMUM Size** **160 litres** (Equivalent size of bath is 50 US and 41 CDN gallons)	**MAXIMUM Size** **250 litres** (Equivalent size of bath is 74 US and 62 CDN gallons)
3%	30 grams	4,800 grams (4.8 kg, 10.58 lbs)	7,500 grams (7.5 kg, 16.5 lbs)
4%	40 grams	6,400 grams (6.4 kg, 14.1 lbs	10,000 grams (10 kg, 22.05 lbs)
5%	50 grams	8,000 grams (8.0 kg, 17.64 lbs)	12,500 grams (12.5 kg, 27.56 lbs)

For a formalin footbath, concentrations of 2 to 5% are recommended.

Table 3. Calculations for a Formalin Footbath (using Formalin, which is 37% Formaldehyde dissolved gas)

		Calculations for the Recommended Footbath Sizes	
Concentration in footbath To make:	mLs of chem./ treatment to add per litre of water in footbath	**MINIMUM Size** **160 litres** (Equivalent size of bath is 50 US and 41 CDN gallons)	**MAXIMUM Size** **250 litres** (Equivalent size of bath is 74 US and 62 CDN gallons)
2%	20 mLs	3.2 litres	5.0 litres
3%	30 mLs	4.8 litres	7.5 litres
4%	40 mLs	6.4 litres	10 litres
5%	50 mLs	8.0 litres	12.5 litres

Cautions regarding Formalin—examples of what should be considered for inclusion on prescriptions:

- Formalin (formaldehyde) is a proven carcinogen.
- Formalin is not effective when ambient temperatures are below 50°F (10°C).
- Cows with open foot sores or lesions should not go through a formalin footbath.
- Formalin must be used in a well ventilated area and eye protection must be worn when handling or pouring.
- Formalin must be securely stored away from access by other animals or people.

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4. Change the Footbath Solution Completely Every 150 (dirty feet) to 250 (clean feet) Cow Passages.

Footbath solutions must be replaced to maintain activity against BDD. The frequency of replacement depends on the product used and the amount of organic material added to the bath by the cows in bathing. The standard recommendation of 150 to 250 cow passages is an estimate of the life of a footbath, as there is very little product specific information available.

One study done on 18 Dutch farms estimated that a 4% formalin footbath dropped to 2% (the lowest effective concentration) after an average of 300 to 320 cow passages. Of the 18 footbaths studied only 11 had an initial concentration of over 2%, even though the producers were targeting 4% to begin the time period under study.

Footbaths work to reduce BDD but careful attention to protocols is needed to make them worthwhile. Done incorrectly, they can damage feet (too high a concentration of formalin) or spread BDD further (too low a concentration allows the bath itself to contaminate the feet of all cows).

Veterinarians providing prescriptions for footbaths must ensure that they include all the information a producer needs to make footbaths that are therapeutically successful, while also ensuring that footbaths are used in a manner that keeps animals and people safe.

Violative Milk Residues Associated with Topical Antibiotic Treatment for Bovine Digital Dermatitis

Gerard Cramer, Cramer Mobile Veterinary Services, Stratford, Ontario

Recently, there have been reports of positive bulk tank milk tests for tetracycline from herds where cows were treated topically for Bovine Digital Dermatitis (BDD). Results of the investigations of these cases are not known, however the occurrence of positive tests has led to considerable discussion and concern regarding the use of tetracycline for foot treatment at the time of hoof trimming.

Antibiotics can potentially contaminate bulk milk after topical treatment of BDD by two routes: either by absorption into the blood stream or by "washing" into the milk from teat skin surfaces. Cows may contaminate teat skin during foot bathing if splashing occurs, or when lying if teat skin comes into physical contact with treated feet or bandages.

All use of topical antibiotics for BDD are extra-label treatments, but veterinarians currently have no pharmacokinetic data to use to make recommendations for appropriate withdrawal periods for milk or meat. To my knowledge, to date there are only two published studies of milk testing following topical antibiotic BDD treatment. In one study, oxytetracycline (liquid) was used for BDD treatment and cows were subsequently tested for the violative residues in milk by two tests: a high pressure liquid chromatography (HPLC) and a rapid bulk milk test, the Charm II. In this study the feet of 28 cows with BDD were treated by spraying for seven days (16 cows) or with a single topical treatment of oxytetracycline covered with a foot wrap (12 cows). None of the samples were found positive on the Charm II (reported test detection level 75 to 100 ppb). The highest level of oxytetracycline by HPLC was 12 ppb. This is well below the maximum residue level (MRL) in Canada for oxytetracycline of 100 ppb. In a similar Ontario study, 20 cows were treated with a paste of tetracycline HCL powder, with or without a foot wrap. Milk from treated cows was tested 24, 48 and 72 hours post-treatment using a rapid bulk milk test; the IDEXX Snap Tetracycline test (reported detection level of 50 ppb). None of these cows had positive tests.

While the literature evaluating residue risk by

(Continued on page 17)

absorption of antibiotics following topical foot treatment is scant, it does so far suggest that the risk of violative tetracycline or oxytetracycline residues, under the studied conditions, is unlikely. The question remains – what can happen on a farm that causes positive bulk tank tests following treatment? Both studies discussed earlier used approximately 2,000 mg of tetracycline per treatment. From discussions with veterinarians and hoof trimmers, it seems likely that much higher doses are commonly being used in the field. While the minimum inhibitory concentration (MIC) for the tetracycline family of antibiotics may be high compared to some other antibiotics, it is highly likely that the concentrations used in the field are still far in excess of what is needed. It is possible that at very high dosages the potential for violative residues increases. Other factors that could affect (increase) antibiotic absorption are the amount of tissue damage in the treated area and the size of the skin area to which treatment is applied. No studies have addressed these factors and their potential impact remains unknown. It seems reasonable that aggressively debrided foot lesions could have an increased likelihood of absorption. Lastly, the possibility of direct contamination of other areas of skin always exists with a topical treatment. Care must be taken to minimize the contamination of the leg, abdominal, teat and udder skin areas during treatment application. Proper udder preparation procedures prior to milking will provide additional insurance by ensuring that any contamination is removed from the teats prior to milking, minimizing this risk.

Further research is planned to address some of the questions that remain. However, until the study is completed, veterinary practitioners must consider all aspects of topical BDD treatment when making recommendations and writing prescriptions. All antibiotic use for treatment of BDD is extra-label as no antibiotics are labeled for use by this route for this condition – therefore such treatment can only be done under veterinary prescription and oversight.

Veterinarians can only provide prescriptions for this use when they are sure that animal and food safety will not be compromised, provided the producer and their hoof trimmer follows the directions as set out in the herd veterinarian's prescription.

Based on the currently available data, it appears that the risk of antibiotic residues due to absorption is low if:

1. The dosage of tetracycline used is measured and appropriate (i.e. use less than <5g of active ingredient).
2. Debridement of tissue is kept to a minimum (clean the lesion but do not cut skin).
3. No bandage chewing occurs (when this occurs, it likely indicates debridement was too aggressive).
4. Care is taken to minimize contamination of the udder and legs at the time of treatment application.

It is essential that veterinary prescriptions caution producers to employ excellent pre-milking udder hygiene (proper milking preparation) to ensure all topical therapeutic, chemical and disinfectant products are removed from teat skin prior to milking.

Britt JS, Carson MC, von Bredow JD, Condon RJ. Antibiotic residues in milk samples obtained from cows after treatment for papillomatous digital dermatitis. J Am Vet Med Assoc 1999; 215(6):833-836.

Cramer G, Higginson J, Kelton DF, Godkin A. Lack of tetracycline residues with two different methods of topical tetracycline treatment for digital dermatitis. Proceedings of 16th Symposium and 8th Conference on Lameness in Ruminants, Rotorua, NZ. 2011: p 7.

Evans NJ, Brown JM, Demirkan I, Birtles R, Hart CA, Carter SD. In vitro susceptibility of bovine digital dermatitis associated spirochaetes to antimicrobial agents. Vet Microbiol 2009; 136 (1-2):115-120.

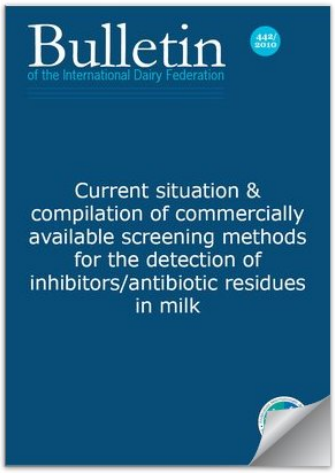
The Dairyland Initiative—The Guide to Welfare Friendly Dairy Cattle Housing

The program was developed by Drs. Nigel Cook and Ken Nordlund at the School of Veterinary Medicine, University of Wisconsin-Madison.

"Optimal dairy cow health and performance happens when she is given an optimal physical, nutritional and managerial environment in which to live and produce."

For further information, visit their website at *http://thedairylandinitiative.vetmed.wisc.edu/index.htm*

Bulletin of the IDF No. 442/2010—Current situation and compilation of commercially available screening methods for the detection of inhibitors/antibiotic residues in milk



- Date: 2010
- Pages: 164
- Paper: 100.00€ (approximately $133.11, December 28, 2011)
- Electronic: 94.00€ (approximately $125.12. December 28, 2011

To view the abstract or order on-line, refer to
www.fil-idf.org/Public/PublicationsPage.php?ID=27121#list
(Type in "Bulletin 442/2010" in the search box and check Bulletin.)

Product Use on Organic Livestock Farms

Websites of interest when considering product use on organic livestock farms:

The Organic Production Systems standards (CAN/CGSB 32.211) on the web:
CAN/CGSB-32.310-2006 Organic Production Systems - General Principles and Management Standards
www.tpsgc-pwgsc.gc.ca/ongc-cgsb/programme-program/norms-standards/internet/bio-org/principes-principles-eng.html

Suggested reading: Section 6.5—Breeding *and* Section 6.7—Livestock Health Care

Permitted Substances Lists (within the Organic Standards):
CAN/CGSB-32.311-2006 OrganicProduction Systems - Permitted Substances Lists
www.tpsgc-pwgsc.gc.ca/ongc-cgsb/programme-program/norms-standards/internet/bio-org/permises-permitted-eng.html

Suggested reading:
Section 5—Permitted Substances for livestock production
(Please note that inclusion on this list does not imply a zero withholding time for meat or milk, nor does it permit extra-label use without a veterinary prescription.)
Section 7—Permitted Substances – Lists for Cleaners, Disinfectants and Sanitizers

Animal Health Care Products and Production Aids

CAN/CGSB 32.311 permits the use of Health Care Products and Production Aids in Table 5.3 under the various categories - Anti-inflammatories, Biologics (including vaccines), Homeopathic and Biotherapies, Parasiticides and Anti-microbials, Plant oils. This document provides a more complete listing of substances commonly used in organic livestock husbandry, along with some specific recommendations of acceptable practices.
www.inspection.gc.ca/food/organic-products/standards/animal-health/eng/1327935670685/1327935800942

The Dairy Practices Council—Information for Sale

Guidelines published by The Dairy Practices Council are now available in the printed version or the digital version (PDF) from their website *www.dairypc.org/catalog/guidelines*

Guidelines available include:

- DPC Guideline Sets
- Animal Health and Care
- Barns
- Bulk Tanks (Farms)
- Butterfat
- Cleaning & Sanitizing
- Cooling
- Dairy Laboratory Testing
- Flavor
- HACCP
- Manure Management
- Milking Machines
- Milking Parlors
- Plant
- Sampling
- Small Ruminants (goats & sheep)
- Somatic Cell
- Tank Trucks
- Waste Management
- Water

Tools for Trouble Shooting SCCs in Ontario, June 12, 13, 14 ..... June 26, 27 & 28

As of August 1, 2012, the Ontario SCC limit will change from 500 to 400K. Many of your clients need to lower counts and prevent mastitis. You have an opportunity to be involved in trouble-shooting mastitis problems!

"From DFO - 19.6% of monthly bulk tank SCCs were greater than 400k in August 2011"
Are you prepared to help them?

Update your skills at a "Tools for Trouble Shooting SCCs Workshop". Presented by members of the Industry SCC 2012 Task Force including: David Kelton and Dan Shock (Ontario Veterinary College), Marc Lazenby and George MacNaughton (Dairy Farmers of Ontario), Richard Cantin and Melanie Quist (CanWest DHI), Ann Godkin (OMAFRA), Jim Fairles (Animal Health Laboratory) and Karen Hand (SSG Solutions).

Topics include:

- The Ontario SCC system – where do your clients fit?
  Update on SCC data and programs
- Motivating producers - Why fix the problem?
  New info on costs due to milk loss from increased SCCs
- Systematic SCC problem solving – the RAMP approach for mastitis
- Monitoring SCCs – Twelve Dairy Comp Tips for SCCs Case studies…hone your approach and apply new skills

Dates and Locations:

Tuesday	June 12	Listowel	*Listowel Golf and Country Club*
Wednesday	June 13	Walkerton	*Dunkeld Restaurant*
Thursday	June 14	Woodstock	*Woodstock OMAFRA Office*
Tuesday	June 26	Maxville	*Maxville Sports Complex*
Wednesday	June 27	Kemptville	*WB George Centre*
Thursday	June 28	Cobourg	*Best Western Cobourg*

All workshops run 10:00 a.m. to 3:00 p.m.. Lunch is included.
Cost: $20.00 per attendee.
Pre-registration required by 7 calendar days ahead of workshop date.
To pre-register: Contact Ann Godkin—ann.godkin@ontario.ca or (519) 846-3409

Continuing Education/Coming Events

June 3-8, 2012	XXVII World Buiatrics Congress, Lisboa Congress Centre, Lisbon, Portugal *wbc-2012.com/index.php/en_US/home*
June 5-7, 2012	3rd International Symposium on Beef Cattle Welfare, Delta Bessborough Hotel, Saskatoon, Saskatchewan. *www.beefwelfare2012.ca*
June 6-8, 2012	World Pork Expo, Iowa State Fairgrounds, Des Moines, Iowa. *www.worldpork.org*
June 12, 13, & 14, 2012	Tools for Trouble Shooting SCCs Workshop (See page 19) June 12, Listowel Golf and Country Club, Listowel, Ontario June 13, Dunkeld Restaurant, Walkerton, Ontario June 14, Woodstock OMAFRA Office, Woodstock, Ontario
June 10-13, 2012	22nd International Pig Veterinary Society Congress—Happy Pigs - Healthy People, International Convention Centre, Jeju, South Korea. *www.ipvs2012.kr*
June 19 & 20, 2012	39th Annual Ontario Pork Congress, Stratford Rotary Complex, Stratford, Ontario *www.porkcongress.on.ca*
June 26, 27 & 28, 2012	Tools for Trouble Shooting SCCs Workshop (See page 19) June 26, Maxville Sports Complex, Maxville, Ontario June 27, WB George Centre, Kemptville, Ontario June 28, Best Western Cobourg, Cobourg, Ontario
July 11-14, 2012	64th Canadian Veterinary Medical Association (CVMA) Convention, Fairmont the Queen Elizabeth Hotel, Montreal, Quebec. *www.canadianveterinarians.net*
July 29-August 2, 2012	17th International Congress on Animal Reproduction (ICAR), Vancouver Convention Centre, Vancouver, British Columbia. *www.icar2012.com/8*
August 9, 2012	Equine Musculoskeletal Ultrasound Wet-lab presented by Central Canada Veterinary Association, horse barns, Kemptville College, Kemptville, Ontario. Presenter will be Dr. Stephanie Nykamp, followed by a hands-on lab session on live horses. Contact Jan Shapiro—jshapiro@kemptvillec.uoguelph.ca
September 20-22, 2012	45th Annual Conference of the American Association of Bovine Practitioners, meeting jointly with the American Association of Small Ruminant Practitioners, Montreal Convention Centre, Montreal, Quebec. *www.aabp.org/meeting/conference.asp*
October 24-26, 2012	Dairy Cattle Welfare Symposium, Delta Guelph Hotel and Conference Centre, Guelph, Ontario. *www.dairycattlewelfaresymposium.ca*
October 25 & 26, 2012	Central Canada Veterinary Association Fall Conference, Strathmere Inn, North Gower, Ontario. The bovine speaker for both days is Dr. Wm Dee Whittier, Director of Veterinary Extension, Bovine Extension Specialist, Virginia-Maryland Regional College of Veterinary Medicine. Contact Jan Shapiro—jshapiro@kemptvillec.uoguelph.ca
November 14 & 15, 2012	Ontario Association of Bovine Practitioners (OABP) Fall Continuing Education Program, Holiday Inn, Guelph, Ontario. *www.oabp.ca*
December 1-5, 2012	58th Annual Convention of the American Association of Equine Practitioners, Anaheim, California. *www.aaep.org/convention.htm*
January 27-29, 2013	National Mastitis Council 52nd Annual Meeting, Omni Hotel, San Diego, California. *www.nmconline.org*
February 18-22, 2013	International Sheep Veterinary Congress, Christchurch Convention Centre, Christchurch, New Zealand. *http://conference.intsheepvetassoc.org*

Mastitis 3 PCR Assay Applied to DHI Preserved, Composite, Metered Samples —Frequently Asked Questions and Answers

Ann Godkin, Veterinary Science and Policy Unit, OMAFRA
David Kelton, Ontario Veterinary College, University of Guelph

1. Why did my milk culture results and PCR results differ?

Research from five Ontario dairy farms with a history of endemic *Staphylococcus aureus* (*SA*) mastitis has shown that PCR results from DHI samples and bacteriological culture results from hand-stripped, composite samples agreed in most cases for the major contagious pathogens, *Staphylococcus aureus, Streptococcus agalactiae* and *Mycoplasma bovis*. Where they disagreed, the differences were usually readily explained.

A PCR result from a test on a DHI milk sample can differ from a routine bacteriological culture result for several reasons. They differ because:

- These PCR and culture are two different kinds of tests and test for different aspects of bacteria.
- The tests are done on different samples, and
- The two tests have a different spectrum of possible results.

Table 1 gives important and specific details about how the samples and tests differ.

2. Why did a cow with a low SCC have a positive *Staphylococcus aureus* (*SA*) PCR result?

A positive PCR result indicates that DNA from *SA* was present in the DHI metered milk sample that was tested. Positive PCR test results from cows with elevated SCCs don't surprise us as *SA* is a frequent cause of subclinical mastitis. We may be more surprised to get a positive PCR result from a cow with a low SCC, but it still makes sense. When cows with high SCCs are tested and the *SA* PCR is positive, it is highly likely that an active, established infection with *SA* is present in the cow tested. After all, if we pick high SCC cows to test, this is a population of cows we already know by their SCCs are highly likely to have some kind of infection present. If, on the other hand, cows are selected for testing by some other criteria, i.e. all cows are tested on first DHI test, or all purchased cows are tested or the whole herd is tested at one time, then, within these groups of cows, we are now testing cows that have both high and low SCCs. In other words, there are now cows being tested that have "mastitis" and cows that have no prior evidence of mastitis. When cows with low SCCs are tested, it's not surprising that some are detected with *SA* DNA. What we are revealing is the population of cows that are exposed to *SA* but in whom it may be an early infection (not yet established, hence no SCC response), where infection is only transitory (it doesn't "take" for whatever reason) or where it is only a teat reservoir or streak canal inhabitant (not an infection of glandular tissue). In all these situations, *SA* DNA is present but no reaction in the cow (elevated SCCs) is present at the same time.

We need to be aware that, in herds with poor milking technique, many cows in the herd are repeatedly challenged at the teat end with *SA* that is circulated at milking time from the chronically infected *SA* cows that are being milked in the herd. Poor milking hygiene, lack of a milking order, poor teat dipping, etc., allows this to happen. Many times, when cows are exposed to *SA* at milking time, infection does not occur, even though small numbers of organisms may be present in milk from the quarter(s) at a given time. Herds with a high prevalence of cows infected with *SA* and who have less than ideal milking procedures are more likely to have some cows with low SCCs that test positive with the Mastitis 3 test. This suggests that *SA* is likely circulating in an unrestricted manner among the udders of the cows milked in the herd.

Decisions about treatment or culling should not be based solely on the outcome of any diagnostic test.

(Continued on page 22)

The Mastitis 3 result is one piece of information that can be added to the cow's mastitis history, her current and historical SCCs, CMT results and other health information, to decide what action, if any, should follow.

Before setting up a sampling and testing protocol, make sure you know the question you want to answer with your test results. Ensure that the test results you receive provide the information you need.

3. Why did a cow with clinical mastitis have a positive *SA* PCR result?

While *SA* is most commonly associated with subclinical infection, it is also an important cause of clinical mastitis. *SA* was the bacterial pathogen most frequently cultured from clinical mastitis cases in herds where all clinical cases were cultured as part of the national CBMRN project [(1)].

The Mastitis 3 PCR Assay tests for the three major contagious mastitis pathogens in herds: *SA*, *Streptococcus agalactiae* and *Mycoplasma bovis*. DHI samples are composite samples. It is possible for a cow to have a *SA* infection in one quarter and to also have an infection with another pathogen in another quarter. When *SA* or another contagious pathogen is identified in the milk sample but the history and signalment in the cow differs from what you expected, it may not be appropriate to assume that the contagious bacteria was the only pathogen in that sample. If mastitis pathogens other than the three included in the Mastitis 3 are present in the sample, they will not be identified. All cows with clinical mastitis should be fully assessed (CMT or other tests, clinical exam, etc.) and handled in an appropriate manner following the specific farm protocols.

4. Can I test for other pathogens with DHI samples?

The Mastitis 3 PCR Assay was originally developed to identify a wide spectrum of bacteria in **aseptically** collected milk samples in a diagnostic laboratory setting, not for direct use on milk samples collected via meters. The decision to investigate its use for testing DHI milk samples was made only after investigation into the reliability of the results. When one of the three contagious pathogens (*SA*, *Streptococcus agalactiae* or *Mycoplasma bovis*) is identified by the PCR test in a DHI metered sample, research has shown there is a high probability that the bacterial pathogen originated in the cow's quarter. However, as DHI samples are collected in meters, after milk travels through milking units and hoses, the samples are not aseptically collected. If the full Mastitis 3 Assay were applied, DNA from many more bacteria would be detected. It would be impossible to tell whether these additional bacteria had originated from the cow's udder or had entered the milk sample from skin surfaces, hoses, meters, equipment, or other sources after leaving the teat end. Therefore, we strongly believe it is inappropriate to apply the full range of the Mastitis 3 Assay for testing of DHI metered milk samples. The results would be very difficult to interpret and likely misleading.

5. Is there risk of "carry-over" of milk and DNA in the milking meters?

Carry-over of a small amount of milk from one DHI cow sample to the next at the time of collection is possible due to residual milk in the milking unit, milk meter or milk sampler. It could also occur in the laboratory if small amounts of milk were carried from one sample to the next on the pipetting equipment used to process the DHI milk samples. However, given the degree of mechanization involved in both sample collection and testing, there is a very small possibility that any carry-over occurs. Based on several investigations carried out in the field and in the DHI laboratory, we are confident that carry-over is unlikely. If it were to occur, the volume of sample potentially carried forward, either in the field or in the laboratory, is so small that dilution would markedly decrease the concentration of DNA to below the PCR detection limits. Therefore, false-positive tests on the Mastitis 3 PCR are unlikely.

6. What is the value of the Beta-Lactamase (βL) result?

Beta-Lactamase is an enzyme that some bacteria produce to inactivate Beta-lactam drugs such as penicillin. Mastitis research has shown that the presence or absence of βL predicts the likelihood

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Table 1. Comparison of Bacteriological Culture and Mastitis 3 PCR Testing of Cow Milk Samples

	Bacteriological Culture of Milk	Mastitis 3 PCR Testing of Milk
Sample Type	Hand stripped sample of one or more (composite usually all 4) quarters Sterile sample – alcohol prep of teat ends required Foremilk only. Collected after milking prep, alcohol prep of teat ends and before milking unit applied.	Collected by meter at one milking Very clean but non-sterile sample Composite samples. Collected from all quarters milking and throughout milk flow
Sample Collection	Collected specifically for culture (only use). Stringent sample collection requirements.	Routinely collected as part of the regular DHI milk recording and testing service.
Sample Handling	Samples must be cooled and arrive at the laboratory within 24 hours of collection to prevent bacterial multiplication and overgrowth of non-target bacteria.	Preservative added at time of sample collection so bacterial multiplication is arrested. Samples are robust to storage and handling conditions.
Volume of Sample Tested	0.1 mL (100 µl) of milk is streaked on the culture plate(s).	0.35 mL (350 µL) of milk is used for DNA extraction and testing.
Pathogen Detection	Bacteria are grown on culture media and identified using a standard protocol for colony identification. Additional tests applied as needed.	Bacterial DNA extracted and identified using real-time PCR.
Range of Pathogens Identified	Broad (all bacteria in milk) – but does not include all mastitis pathogens (i.e. *Mycoplasma bovis* requires specific media for growth and identification).	Mastitis 3 is a very specific test. The test detects only *Staph. aureus*, *Strep. agalactiae* and *Mycoplasma bovis*. Even if other mastitis bacteria are present they will not be identified and the test will not be positive.
Identification of β-lactamase producing *Staph. aureus*	*Staph. aureus* colonies are picked and specifically tested for β-Lactamase production capability.	Presence of a Staphylococcal β-lactamase gene in the milk sample identified by PCR.
Classifying sample as contaminated	Based on identification of 3 or more bacterial species in one sample.	Not possible, given the limited number of bacteria identified and sample type.
Laboratory time from sampling to results	Days. Can vary depending on additional tests required for identification.	Hours. Always the same test protocol.
Laboratory cost (testing)	$8 per sample	$24 per sample

of success of a wide variety of antibiotics, not just penicillin. βL testing is usually done in a conventional bacteriology laboratory directly on colonies identified as *SA*. The Mastitis 3 PCR test tests for the presence of the βL producing gene in any of the bacteria in the milk sample, not just *SA*. Unfortunately, we cannot tell if a positive PCR test means the βL gene is in *SA* in the same sample or in some other unidentified and non-mastitis bacteria that are also present. Given this limitation, there is no added value from the βL result for making therapy decisions under the current test conditions.

7. Why was a cow's PCR result positive one month and negative the next?

A cow may be PCR-positive at one point in time and PCR-negative at the next test, regardless of whether that next test is days, weeks or months later, for several reasons. Cows infected with *SA* may shed *SA* in the milk in low numbers and intermittently, thus the amount of DNA in the milk from an infected cow can vary dramatically from day to day, and may fluctuate above and below the detection limit of the PCR test.

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The second reason for a negative test following a positive test is that it is possible for cows to cure *SA* infection either spontaneously or following antibiotic therapy. While we know that cure rates for well established *SA* infections are very, very low, it is possible for some infections to be only temporary. These *SA* infections resolve on their own or as a result of antibiotic therapy.

Olde Riekerink RGM, Barkema HW, Kelton DF, Scholl DT. Incidence Rate of Clinical Mastitis on Canadian Dairy Farms. J Dairy Sci 2008; 91 (4):1366-1377.

Ceptor Animal Health News
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Ontario

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