

DAIRY VETERINARY NEWSLETTER

January 2018

Freezing and Thawing Bovine Colostrum and Survival (Isolation) of Mycoplasma over Time

An interesting short communication paper by L. Gille et al. was published in the January 2018 issue of J Dairy Sci. Mycoplasma has been found in colostrum. The authors stated, “Colostrum has been [reported] as a possible source of [mycoplasma in calves] infection, but the prevalence of *M. bovis* in colostrum samples is currently unknown (Godden et al., 2006). - - The presence of *M. bovis* in colostrum is currently unaccounted for in *M. bovis*-preventive protocols.”

In my experience, mycoplasma management protocols do often recommend only feeding colostrum from cows tested negative for mycoplasma if the disease has been detected in adult cows in the herd, but many producers do not strictly follow this advice, if at all. Cows that are test-negative in a herd where some adult cows are mycoplasma-positive in milk may only become detected positive later. They may intermittently shed mycoplasma at only some milkings, especially just after calving, before their subsequent detection as positive. It has been demonstrated that sensitivity of detection of adult cows with mycoplasma in milk is highest during the first 21 days in milk, but often when adult cows are cultured for mycoplasma, the sampling is done such that few recently calved cows are tested. The collection of samples is often done with many or all lactating cows tested at the same time, thus by probability most of them are not in early lactation, and may calve again before further testing.

We have had excellent success (94% of herds with mycoplasma detected in cows became consistently test-negative for mycoplasma in milk of all cows within 2 years, and remained so for 8 to 10 years now) using a test and cull strategy. However, until mycoplasma is eliminated from the adult herd, colostrum may be positive for *Mycoplasma* spp.

The authors wanted to evaluate survival of mycoplasma in frozen colostrum to try to answer 2 questions:

- Does freezing colostrum for various times and temperatures, and thawing at various temperatures affect the ability to isolate/detect *M. bovis* by decreasing its concentration in colostrum?
- Can the above practices eliminate *M. bovis* in colostrum?

The study did not use naturally infected colostrum. Instead, gamma irradiated sterile colostrum - verified by culture on PPLO agar for 7 days - was inoculated with one of 2 strains of *M. bovis*. Strains were from a bronchoalveolar lavage sample from a calf with pneumonia, and from milk of a cow with mastitis, arthritis, and an infected seroma.

Briefly, *M. bovis* was confirmed by PCR, suspended in a storage medium of horse serum, brain heart infusion broth, and glucose and inoculated into 10 ml of modified PPLO broth. Broth was incubated for 5 d at 35°C and 5% CO₂, centrifuged, and resuspended in sterile PBS. Mycoplasma were diluted to an initial concentration of 10¹⁰ cfu/ml of *M. bovis* using an optical density meter, and then further diluted 1:10,000 to 10⁶ cfu/ml of *M. bovis* in colostrum. In my experience, using some indirect measure of concentration and then diluting from there may not result in cultures of various dilutions of the

final product indicating the concentration expected. Therefore it was good that the authors verified the initial concentrations of mycoplasma in colostrum:

Colostrum (100 ml) was plated on PPLO agar “using serial dilutions. Samples were incubated for 1 wk at 35°C and 5% CO₂, and colonies (with the typical fried-egg appearance) were counted with help of a microscope (10× magnification).” There were some complex statistical methods used, but the outcome variable was not stated. (Brief communications don’t leave room for every detail.) In one experiment, I think the outcome variable was concentration of *M. bovis* in cfu/ml of colostrum. A linear mixed model with repeated measures was used to evaluate factors affecting the outcome (again, it seemed to be cfu/ml of *M. bovis* in colostrum) at inoculation, and after freezing colostrum for 1, 4, 10, and 14 weeks, compared among the 2 strains. Alpha for significance was $P < 0.05$.

Another experiment tested “effect of repeated thawing (once or twice) and temperature of the first thawing [either 20°C (room temperature) or 45°C] on *M. bovis* recovery” from colostrum. (Only the calf pneumonia strain was tested.) This time two starting concentrations, 10⁶ cfu/ml and 10⁴ cfu/ml of mycoplasma were evaluated. Tested processes were:

- freezing (-18°C) for 2 wk and a single thawing at 20°C
- freezing for 2 wk and a single thawing at 45°C
- freezing for 1 wk and thawing at 45°C, refrozen for 1 wk and thawing at 20°C
- freezing for 1 wk and thawing at 20°C, refrozen for 1 wk and thawing at 20°C

In the first experiment, for both mycoplasma strains, freezing for 1 wk reduced the concentration of mycoplasma in colostrum by 1 log, from 10⁶ cfu/ml to approximately 10⁵ cfu/ml. The longer freezing times up through 14 wk did not further reduce mycoplasma concentration.

In the second experiment, for either starting concentration (10⁴ or 10⁶ cfu/ml), “freezing and thawing of *M. bovis*-infected colostrum reduced the [mycoplasma] concentration by approximately 1 log compared with the initial concentration. Thawing temperature (20 vs. 45°C) did not have a significant effect on the survival of *M. bovis* in colostrum (P = 0.43). Repeated thawing of colostrum further decreased the number of *M. bovis* by approximately 0.5 log compared with single thawing, regardless of the starting concentration of *M. bovis* (P < 0.05).”

Practicality of long-term freezing of colostrum

As the authors point out, mycoplasma concentration in milk has been reported between 10² and 10⁸ cfu/ml. The infectious dose of total mycoplasma bacteria has been speculated to be 100 cfu or less. It would be interesting to see how the freezing times and thawing temperatures affect colostrum with a starting mycoplasma concentration of 10² cfu/ml. Also freezing at commercial freezer temperatures, usually -1 to -4°C, would be of interest. The authors state two major points of practicality from these results:

- For purposes of research sample storage or on-farm monitoring of colostrum samples for mycoplasma, either for calf feeding decisions or possible culling of dams, freezing (-18°C) for up to 14 wk has little impact on mycoplasma detection in colostrum.
- “These results - - illustrate that long-term freezing of colostrum is not a valid control strategy to prevent *M. bovis* infection of neonatal calves. - - multiple freeze-thaw cycles can cause an undesirable decline in maternal antibody levels (reference provided in full paper) and are thus not recommended.”

New Schedule of Services and Fees at the Utah Veterinary Diagnostic Laboratory (next 3 pages)

Fee Structure January 1, 2018

A \$10.00 accession fee is applied to each submission except for state/federal program disease serologic testing and bulk bovine pregnancy testing.

Bacteriology

Aerobic culture	\$15.00/ea.
Anaerobic culture	\$20.00/ea.
Antimicrobial sensitivity	
Companion animal	\$17.50/ea.
Food animal	\$15.00/ea.
Poultry	\$15.00/ea.
<i>Campylobacter</i> spp.	\$15.00/ea.
Fungal culture	\$20.00/ea.
<i>Listeria</i> spp.	\$20.00/ea.
Milk culture	\$5.00/ea.
Milk culture, bulk tank	\$20.00/ea.
RODAC plate	\$12.50/ea.
<i>Salmonella</i> spp.	
Culture	\$17.50/ea.
RapidChek Group D	\$17.50/ea.
Total plate count	\$45.00/ea.
Water culture	\$20.00/ea.

Clinical pathology

Albumin	\$15.00/ea.
Bilirubin, total	\$15.00/ea.
Cerebral spinal fluid	
Glucose	\$15.00/ea.
Protein	\$20.00/ea.
CO ₂	\$15.00/ea.
Creatine kinase	\$15.00/ea.
Cytology	\$30.00/ea.
Fluid panel	\$37.00/ea.
Endocrine	
ACTH stimulation	\$50.00/ea.
Cortisol	\$26.00/ea.
Dexamethasone suppression	\$75.00/ea.
T4, free	\$35.00/ea.
T4, total	\$25.00/ea.
Progesterone	\$25.00/ea.
Thyroid, profile	\$85.00/ea.
TSH	\$35.00/ea.
Fecal occult blood	\$5.00/ea.
Fibrinogen	\$5.00/ea.
Hematology	
Blood film review	\$15.00/ea.
CBC, mammalian	\$25.00/ea.
CBC, non-mammalian	\$30.00/ea.
Hemogram	\$15.00/ea.
PCV & plasma protein	\$8.00/ea.
Platelet count	\$17.00/ea.
Reticulocyte count	\$17.00/ea.
Liver	
Alkaline phosphatase	\$15.00/ea.
ALT	\$15.00/ea.
AST	\$15.00/ea.
Avian hepatic profile	\$30.00/ea.
Bile acids	\$30.00/ea.
GGT	\$15.00/ea.
Liver profile, large animal	\$30.00/ea.
Liver profile, small animal	\$30.00/ea.
SDH	\$15.00/ea.

Metabolic testing

β-hydroxybutyrate	\$25.00/ea.
Cholesterol	\$15.00/ea.
Glucose	\$15.00/ea.
Profile, large animal	\$45.00/ea.
Protein, total	\$15.00/ea.
Triglycerides	\$15.00/ea.
Phenobarbital	\$35.00/ea.
Serum chemistry	
Avian/reptile chemistry	\$45.00/ea.
Chloride	\$15.00/ea.
Large animal panel	\$45.00/ea.
Large animal panel w/ lipids	\$70.00/ea.
Magnesium	\$15.00/ea.
Phosphorus	\$15.00/ea.
Potassium	\$15.00/ea.
Small animal panel	\$35.00/ea.
Sodium	\$15.00/ea.
Urinary	
BUN	\$15.00/ea.
Creatinine	\$15.00/ea.
Renal profile	\$25.00/ea.
Uric acid	\$15.00/ea.
Urinalysis	\$20.00/ea.
Urine cortisol/creatinine	\$35.00/ea.
Urine GGT	\$15.00/ea.
Urine potassium	\$15.00/ea.
Urine protein	\$20.00/ea.
Urine protein/creatinine	\$30.00/ea.
Urine sodium	\$15.00/ea.

Disposal, cremation, incineration

Cremation (ashes returned)	\$75.00 + \$1.00/lb
Disposal	
< 100 lb	\$30.00/ea.
100–300 lb	\$50.00/ea.
> 300 lb	\$95.00/ea.
Incineration	
< 50 lb	\$50.00/ea.
50–100 lb	\$65.00/ea.
100–200 lb	\$90.00/ea.
> 200 lb	\$0.80/lb

Histology

H&E stained recut.....	\$3.50/ea.
Histochemical special stain.....	\$10.00/ea.
Tissue decalcification.....	\$3.00/ea.

Immunohistochemistry

Chronic wasting disease.....	\$25.00/ea.
Scrapie.....	\$25.00/ea.

Molecular diagnostics

Avian	
Avian influenza–A matrix, single.....	\$35.00/ea.
Avian influenza–A matrix, pool of 5.....	\$7.00/ea.
Avian influenza–H5/H7.....	\$20.00/ea.
Avian paramyxovirus	\$35.00/ea.
Exotic Newcastle disease	\$25.00/ea.
<i>Mycoplasma gallisepticum</i>	\$25.00/ea.
<i>Mycoplasma synoviae</i>	\$25.00/ea.
<i>M. gallisepticum</i> & <i>M. synoviae</i>	\$40.00/ea.
Pigeon virology panel.....	\$35.00/ea.
<i>Salmonella enteritidis</i>	\$30.00/ea.
<i>Salmonella</i> Group D (1–5)	\$35.00/ea.
<i>Salmonella</i> Group D (6+).....	\$30.00/ea.
Cattle	
Bluetongue (1 to 89).....	\$30.00/ea.
Bluetongue (>90).....	\$22.50/ea.
Bovine abortion panel (BVD & IBR).....	\$40.00/ea.
Bovine respiratory panel.....	\$50.00/ea.
BVD.....	\$30.00/ea.
Calf enteric panel.....	\$55.00/ea.
Chlamydiaceae	\$30.00/ea.
Cryptosporidium.....	\$30.00/ea.
IBR	\$30.00/ea.
Johne's disease, pool of 5.....	\$6.00/ea.
Johne's disease, single	\$30.00/ea.
<i>Leptospira</i> spp.	\$30.00/ea.
<i>Mycoplasma bovis</i>	\$30.00/ea.
<i>Salmonella</i> spp.	\$30.00/ea.
<i>Trichomonas foetus</i> , pool of 5*	\$7.00/ea.
<i>Trichomonas foetus</i> , single*.....	\$20.00/ea.

*5% discount for samples submitted in tubes

Horses

Equine herpesvirus–1	\$35.00/ea.
West Nile Virus	\$30.00/ea.
Pan- <i>Mycoplasma</i> spp.	\$25.00/ea.
Sequencing.....	\$50.00/ea.

Parasitology

Cryptosporidium fecal smear	\$12.50/ea.
Fecal float	
Qualitative	\$10.00/ea.
Quantitative.....	\$20.00/ea.
With sedimentation	\$20.00/ea.
Giardia ELISA	\$17.50/ea.
<i>Trichomonas</i> spp. culture	\$7.50/ea.

Pathology

Biopsy		
Single tissue	\$40.00/ea.	
(each additional site \$15.00)		
Field necropsy	\$50.00/ea.	
Brain removal	\$25.00/ea.	
Euthanasia		
Large animal	\$90.00/ea.	
Small animal	\$50.00/ea.	
Avian spp.	\$10.00/ea.	
Necropsy (includes gross, histopathology & disposal; other ancillary tests extra)		
Companion animal	\$120.00/ea.	
Livestock & wildlife		
< 300 lb.....	\$85.00/ea.	
300–1000 lb	\$120.00/ea.	
> 1000 lb.....	\$150.00/ea.	
Horses, camelids, captive cervids, others		
<200 lb.....	\$100.00/ea.	
200–1000 lb	\$150.00/ea.	
>1000 lb.....	\$250.00/ea.	
Poultry & gamebirds		\$60.00
(3 birds; each additional bird \$15.00/ea.)		
Reptiles and small birds	\$45.00/ea.	
Small mammals (hamsters, mice, etc.).....	\$45.00/ea.	
Spinal cord removal		
Equine/companion animals	\$60.00/\$40.00/ea.	

Serology

Avian/Poultry

Influenza type A

AGID	\$6.00/ea.
ELISA, multiple.....	\$3.00/ea.
Antigen-based ELISA (FluDetect)	\$12.00/ea.
<i>Bordetella avium</i> ELISA.....	\$5.50; ≥15, \$3.00/ea.
Encephalomyelitis ELISA.....	\$5.50; ≥15, \$3.00/ea.
Hemorrhagic enteritis ELISA ..	\$5.50; ≥15, \$3.00/ea.
Infectious bronchitis ELISA	\$5.50; ≥15, \$3.00/ea.
Infectious bursal disease ELISA	\$6.00/ea.

Mycoplasma spp.:

Agglutination

<i>gallisepticum</i>	\$5.50; >15, \$3.00/ea.
<i>synoviae</i>	\$5.50; >15, \$3.00/ea.
<i>meleagridis</i>	\$5.50; >15, \$3.00/ea.

ELISA

<i>gallisepticum</i>	\$5.50; >15, \$3.00/ea.
Mg/MS.....	\$5.50; >15, \$3.00/ea.
Paramyxovirus ELISA.....	\$5.50; ≥15, \$3.00/ea.
<i>Salmonella pullorum/typhoid</i> agglutination	\$5.50; ≥15, \$3.00/ea.

Serology (continued)

Cattle	
Anaplasmosis.....	\$9.00; ≥15, \$6.00/ea.
Bluetongue	
AGID	\$8.00/ea.
ELISA	\$7.00; ≥15, \$4.50/ea.
BLV ELISA.....	\$7.00; ≥15, \$4.50/ea.
<i>Brucella abortus</i>	
BAPA.....	\$5.00; ≥15, \$4.00/ea.
Card.....	\$6.00; ≥15, \$4.00/ea.
FPA	\$10.00/ea.
RAP.....	\$5.00; ≥15, \$4.00/ea.
Rivanol.....	\$10.00/ea.
SPT.....	\$6.00; ≥15, \$4.00/ea.
BVD ELISA	\$6.00; ≥15, \$4.00/ea.
Johne's disease ELISA.....	\$7.50; ≥15, \$4.00/ea.
Pregnancy	\$6.50; ≥15, \$2.50/ea.
Dogs	
<i>Brucella canis</i> agglutination.....	\$20.00/ea.
<i>B. canis</i> 2 ME-RSAT.....	\$20.00/ea.
Horses	
EIA AGID	\$12.00/ea.
EIA ELISA	\$10.00; ≥15, \$8.00/ea.
EIA ELISA GVL.....	\$11.00; ≥15, \$9.00/ea.
IgG (foal).....	\$28.50/ea.
Piroplasmiasis	
<i>Babesia caballi</i>	\$20.00/ea.
<i>Theileria equi</i>	\$20.00/ea.
Vesicular stomatitis virus	\$40.00; ≥15, \$30.00/ea.
West Nile virus.....	\$20.00/ea.
Rush Fee.....	\$60.00/ea.
Small ruminants Bluetongue	
AGID	\$8.00/ea.
ELISA	\$7.00; ≥15, \$4.50/ea.
<i>B. ovis</i> ELISA.....	\$7.50; ≥15, \$5.50/ea.
<i>B. melitensis</i> card.....	\$8.00; ≥15, \$6.50/ea.
CAE	\$7.50; ≥15, \$5.00/ea.
Chronic wasting disease ELISA	\$25.00/ea.
Epizootic hemorrhagic disease AGID	\$8.00/ea.
Pregnancy	\$6.50/ea.

Toxicology

Mineral analyses	
29 element screen	\$50.00/ea.
(Ag, Al, As, Ba, Be, B, Cd, Ca, Cr, Co, Cu, Fe, K, Li, Mg, Mn, Mo, Na, Ni, P, Pb, Sb, Se, Si, Sn, Sr, Tl, V & Zn)	
Individual elements	\$20.00/ea.
(includes all elements in the 29 element screen plus Cs, Au, La, Hg, Pt, Re, Rh, Ti & W)	
29 element screen plus individual elements - Contact laboratory	
Mercury & lead.....	\$30.00/ea.
Cadmium, mercury & lead	\$35.00/ea.

Toxicology (continued)

Mercury & selenium	\$30.00/ea.
Nitrate analyses Forage.....	\$20.00/ea.
Water.....	\$15.00/ea.
Ocular.....	\$15.00/ea.
Plant identification.....	\$20.00/ea.
pH	\$10.00/ea.
Water hardness	\$25.00/ea.

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Please let us know your comments and suggestions for future topics. I can be reached at (435) 760-3731 (Cell), (435) 797-1899 M-Tues, (435) 797-7120 W-F or David.Wilson@usu.edu.



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