



# StockQuotes: Animal Health Newsletter

<http://liv.mt.gov/ah/newsletter>

June 2014

Volume 7, Issue 2

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## CALENDAR OF EVENTS:

**VMA Summer Meeting:**  
Jun 29 - Jul 1, Missoula

**Board of Livestock:**  
Jul 29, Helena

**Deputy Veterinarian Training:**  
Sep 25, Helena

## State Veterinarian Notes

In this issue of StockQuotes we discuss an interesting report of heartworm from a veterinarian in Flathead valley. Perhaps this disease has been underdiagnosed based on the perception that our climate is cold enough to keep the risk low. Please report cases of heartworm that you see in your practice to our office.

The Montana Veterinary Diagnostic Laboratory deserves special mention. We're asking your opinion on your use of the veterinary diagnostic laboratory. As a Montana veterinarian, the laboratory you choose to use is extremely important, and we want the Montana laboratory to meet your needs. The short survey is at <http://goo.gl/kP8OVX>.

Don't miss the special section on the growing science of genome analysis of *Brucella* isolates. A recently developed technique is helping us create some links between isolates. This issue covers the basics of the technology and a subsequent issue will discuss Greater Yellowstone Area isolates.

It seems that whenever we think we've caught up on rulemaking, something else comes up. Currently, three rules are open for public comment: 1) Most significantly, we're proposing to adjust the boundary of the DSA (more in the brucellosis column). 2) We're updating the rabies rule to make the DOL rule consistent with public health regulations in how animals that bite humans are managed. We're also taking the opportunity to remove some outdated language regarding 60-day county quarantines that are placed following a rabies diagnosis in a terrestrial animal. 3) Lastly, we're making a non-substantive change to the deputy veterinarian rule by replacing an incorrect refer-

*(Continued on page 6)*

## WHAT'S NEW:

1. Diagnostic Laboratory survey available here: <http://goo.gl/kP8OVX>.
2. Administrative Rules comment period closes July 10.

## Heartworm

DOL receives periodic reports of heartworm from practitioners across Montana. A recent conversation with a veterinarian from north-west Montana raised several significant questions about the disease.

Many confirmed cases of heartworm have a travel history outside of Montana to regions that are considered to have higher prevalence. According to the American Heartworm Society© the incidence in Montana ranges from 1-5 cases per reporting clinic to <1 case per reporting clinic to no data available.

Using a commercially available dog-side antigen test, a reporting practitioner has had 15 dogs test positive for heartworm so far in 2014. None of the animals had a travel history out of Montana. None of the dogs are symptomatic at this time.

All dogs that have tested positive were retested using a test from a different lot, same manufacturer. If the animal was positive a second time (or third or fourth, as was the case for many tested), the blood was shipped to the manufacturer of the test where confirmatory testing was performed.

According to the manufacturer of the test, from 2013 to 2014, there were significant improvements made to the sensitivity of the test (from 84% to 98%) and it's possible that some of these animals may have been positive in past years, but had not been detected. The manufacturer of the test has confirmed all results as positive. The veterinarian intends to retest all animals this fall before beginning any treatment regimen.

Although the attending veterinarian has not performed an exhaustive analysis of the signalment of all test-positive animals, anecdotally there appears to be no connection between animals. They are from a wide geographic range. There is no age, sex, or breed that is predominant. All animals would be classified as indoor/outdoor animals with varying levels of activity.

*(Continued on page 6)*

## Do's and Don'ts of Health Certificates

Import permits for animal entry are issued 24/7 either by DOL import staff, or our contracted after-hours service.

With traceability requirements firmly in place, we've also been reviewing Montana origin health certificates for animals leaving the state. Anne Wheeler, a temporary employee with DOL, enters Montana origin health certificates into the animal health database. She suggested that the following issues with health certificates need special attention.

1. Accreditation code is lacking. Signatures are often impossible to read, and the accreditation code is needed to identify the veterinarian who issued the CVI. Please include it.
2. The term "cow" or "bull" is sometimes used to specify age. "Adult" is more appropriate to indicate an adult animal.
3. The term heifer or heiferette is sometimes used to specify age. This term is ambiguous (heiferette can mean female over 24 months of age) and is especially problematic with the 18 month cutoff for the traceability rule.
4. "Steer" is often written with no age listed.
5. Breed is often missing. In these cases, we default to using "beef" as a breed.
6. Listing too many tags on the CVI can waste your hard efforts. One recent CVI listed 400 individual tag numbers on a single page which made it nearly impossible to read. Keep in mind that we have CVI continuation sheets or you can attach a separate sheet with animal IDs.
7. Please make sure the number of tag numbers listed on CVI matches total number of animals listed in the "total" slot on the certificate. Sometimes the numbers are much different.
8. Some export CVIs are lacking official tag numbers altogether for adult cattle.
9. When the county of origin is within the DSA, a clear statement of whether the cattle originate within the DSA should be made on the export CVI.
10. Some confusion exists between intrastate and interstate. Intrastate movement only need a brand inspection with the CVI being optional. ☒ mz

## New Laboratory Testing Options

**BOVINE VIRAL DIARRHEA VIRUS (BVDV):** The antigen capture ELISA test has been the basis for testing of ear notches to date at a cost of \$5 per sample. Considerable savings can be made when testing larger groups of animals if pooled PCR is requested. Pooled PCR for up to 24 individuals will cost \$50 and provide considerable savings.

Individual ear notch samples should be submitted to the laboratory in 5 ml polypropylene or serum tubes (polypropylene tubes are available from Fischer Scientific cat# 222-2367 \$120 per bag of 500; two position caps cat# 300-3812-02P are approx. \$44/bag of 1000).

Pooling will be carried out at the laboratory. Individual samples in a positive pool will be tested by antigen capture ELISA at \$3.50 per sample.

**JOHNE'S DISEASE:** Both Individual PCR (\$30 per sample) and pooled PCR (\$35 for pool of five samples) procedures are now available.

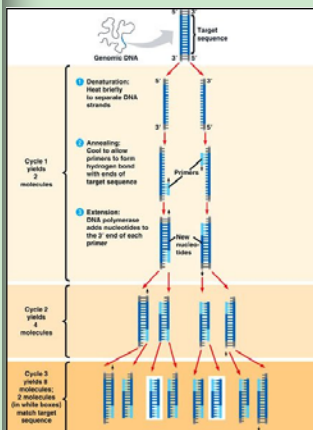
Individual samples (approximately 20 mls of feces) should be submitted in a non-sterile plastic container (eg 80 ml non sterile, unlabeled standard opening, yellow cap, plastic container available from Fischer Scientific cat # 15-182-72A, approximately \$123 for case of 500)

Pooling of up to five samples can be done at the laboratory. You can elect to test individual animals within a positive pool for an additional \$30.

Other important things to remember when submitting samples for PCR testing: PCR is a very sensitive test and subject to erroneous results through contamination. Therefore, samples should be submitted in the containers requested without gross contamination on the outside of the container.

Preg check gloves or plastic bags should not be used as sample containers, if possible. In cases where samples are poorly or inappropriately submitted, significant technician time is often required to prepare samples for testing. An additional handling charge of \$1 per sample may be applied in such cases. ☒

By Jeff Marshall (Pathologist),  
Veterinary Diagnostic Laboratory.



Amplification of nucleic acid through PCR.

Source: <http://bouletsapbiology.edublogs.org/>

# Genotyping 101

New tools are allowing us a closer look at brucellosis transmission and how the disease moves across the landscape.

Traditional epidemiologic investigation methods to determine the source of the infection and the scope of its spread have relied heavily on tracing animal movements and testing potentially exposed animals. Until recently, molecular genotyping methods for *Brucella abortus* have provided insight on the relatedness of infections from various geographic areas; however, this method only examined and compared a small portion of the bacterial genome, giving us an incomplete view of genetic changes and limiting our discriminatory power.

With the advent of lower cost sequencing equipment and supercomputers to manage data, the National Veterinary Services Laboratory (NVSL) is now performing Whole Genome Sequencing (WGS), which examines the entire genome of *B. abortus* isolates.

We know that bacterial replication is imperfect and, over time, replication errors accumulate. WGS tracks and takes advantage of these imperfections as they carry forward in subsequent bacterial generations.

In a nutshell, this technique compares differences in the field strain bacterial genome to that of a known reference *B. abortus* at the base-pair or nucleotide level. This comparison is accomplished using a supercomputer; the data from a single isolate's sequence would extend 2146 miles of 12 point font type. This alignment with a reference strain serves as a "starting point" in bacterial genetic historical terms, allowing us to make comparisons in bacterial genetic changes over time.

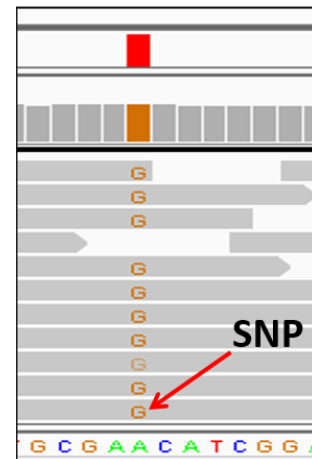
There are many mutations that can occur in a genome as it replicates: insertions, deletions, repeat increases or decreases, nucleotide substitutions. The sequencing method at NVSL only considers nucleotide substitutions, called Single Nucleotide Polymorphisms (SNPs – pronounced "snip").

While replication errors (SNPs) are rare, they do regularly occur within the organism at the rate of 1 mistake per 100 million to 1 billion nucleotides. Given the rarity of replication errors and relative rarity of transmission, we estimate that SNPs occur approximately once for every 1000 generations of bacteria, or roughly one-half of a SNP change every year. Once a SNP is identified, all the bacterial offspring will carry that SNP. These errors usually occur during a period of stress for the bacteria, often called a "bottleneck". One of the most common bottlenecks is transmission between animal hosts.

Figure 1 shows a SNP compared to a reference strain at the nucleotide level. Each grey horizontal line represents a sequenced isolate with the reference isolate listed on the bottom. The output and results of WGS are expressed in SNP difference matrix tables, defining SNPs tables, and phylogenetic trees (Figure 2).

Using these facts and estimated extrapolated time line, we can use SNPs and our local epidemiologic data to further understand how *B. abortus* is moving among and between our animal populations. Next issue, we will explore the results and how they are informing brucellosis eradication efforts. ☒

By Kammy Johnson, DVM, PhD  
USDA-APHIS, Veterinary Services



**Figure 1.** Single nucleotide polymorphisms (SNP) from the reference isolate. In this example, an error in replication substituted Guanine for Adenine in subsequent generations.



**Figure 2.** A larger comparison of SNPs compared to the reference strain. SNPs are called using stringent criteria, and therefore SNPs listed will be highly conserved. Isolates containing the ancestral (reference) genotypes are bolded.

Image credit: NVSL

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<b>reference_call</b>	<b>T</b>	<b>G</b>	<b>C</b>	<b>A</b>	<b>G</b>	<b>T</b>	<b>C</b>	<b>T</b>	<b>T</b>	<b>A</b>	<b>C</b>	<b>C</b>	<b>C</b>	<b>T</b>	<b>C</b>	<b>C</b>	<b>G</b>	<b>T</b>	<b>G</b>	<b>A</b>	<b>C</b>	<b>G</b>	<b>A</b>	<b>G</b>	<b>C</b>	<b>C</b>	<b>G</b>	<b>T</b>	<b>A</b>	<b>C</b>	<b>G</b>	
MT-067_08BA1_CA-08A1_B08-0416	A	T	T	G	C	C	T	G	T	T	A	C	C	C	T	C	C	G	T	G	A	C	G	A	G	C	C	G	T	A	C	G
MT-067_11BA1_CA-11A3_B11-0612	A	T	T	G	C	C	T	G	C	C	G	T	T	C	T	C	C	G	T	G	A	C	G	A	G	C	C	G	T	A	C	G
MT-067_11BA1_CA-11A2_B11-0611	A	T	T	G	C	C	T	G	C	C	G	T	T	A	T	C	C	G	T	G	A	C	G	A	G	C	C	G	T	A	C	G
MT-025_07BA1_CA-07A1_B07-0266	T	G	C	A	G	T	C	T	T	T	A	C	C	C	C	T	T	T	C	A	G	G	A	T	T	C	C	G	T	A	C	G
MT-067_09BA1_BI-WLx1_B09-0553	T	G	C	A	G	T	C	T	T	T	A	C	C	C	C	T	T	T	T	G	A	C	G	A	G	T	T	T	G	C	T	A

# Brucellosis Update

**DSA PROPOSED BOUNDARY CHANGE:** Elk surveillance in 2014 found 10 seropositive elk of 60 sampled in the Black's Ford and Red Mountain area (south of I15 south of Three Forks—see map below left). At the May Board of Livestock meeting, the board approved the publication of a proposed rule that would add this area to the Designated Surveillance Area. This adjustment is necessary to ensure the continued trust that other states place in our DSA regulations.

See the map (below right) for the proposed adjustment. Public comment closes on July 10. The proposed rule and additional information is available on our website here: <http://liv.mt.gov/public/arm.mcp>. DOL will be holding a public meeting at Headwaters Livestock Auction in Three Forks to discuss the proposal at 10 a.m. on July 2.

**BISON QUARANTINE FEASIBILITY STUDY (BQFS):** The goal of the BQFS has been to establish a protocol by which disease-free animals could be selected from the Yellowstone National Park (YNP) herd that is endemically infected with brucellosis at approximately 50%. As part of the BQFS several dozen bison were relocated from YNP to quarantine containment facilities. Following the initial phase of the project, Turner Enterprises hosted 81 Yellowstone Bison for an additional extended surveillance period and these animals, in addition to 65 offspring, are now eligible for transfer to their final destination. The Department of Fish Wildlife & Parks (FWP) published a request

for proposals (RFP) for entities interested in receiving the bison. In early June, proposals from ten groups in several states interested in receiving these bison were reviewed. Applications included tribal entities, state wildlife agencies, and zoos.

In early June, the selection committee narrowed down the possible recipients to the Fort Peck Indian Reservation (MT), Utah Division of Wildlife Resources, Wildlife Conservation Society (NY & OH), Cherokee Nation (OK), and the American Prairie Reserve (MT).

**ENVIRONMENTAL ASSESSEMENT (EA) ON YEAR ROUND BISON:** Also at the May meeting, the Board of Livestock discussed the EA on year-round tolerance for bison in areas adjacent to YNP. This EA was published last fall and has been on the board agenda on two previous occasions. The proposed modified alternative paired the most liberal tolerance in Montana to a population level of 3,000 or less, and scaled-back the available range commensurate with larger numbers. After evaluating the proposal, and receiving feedback from a variety of constituencies the board tabled the EA decision indefinitely.  $\alpha$  mz

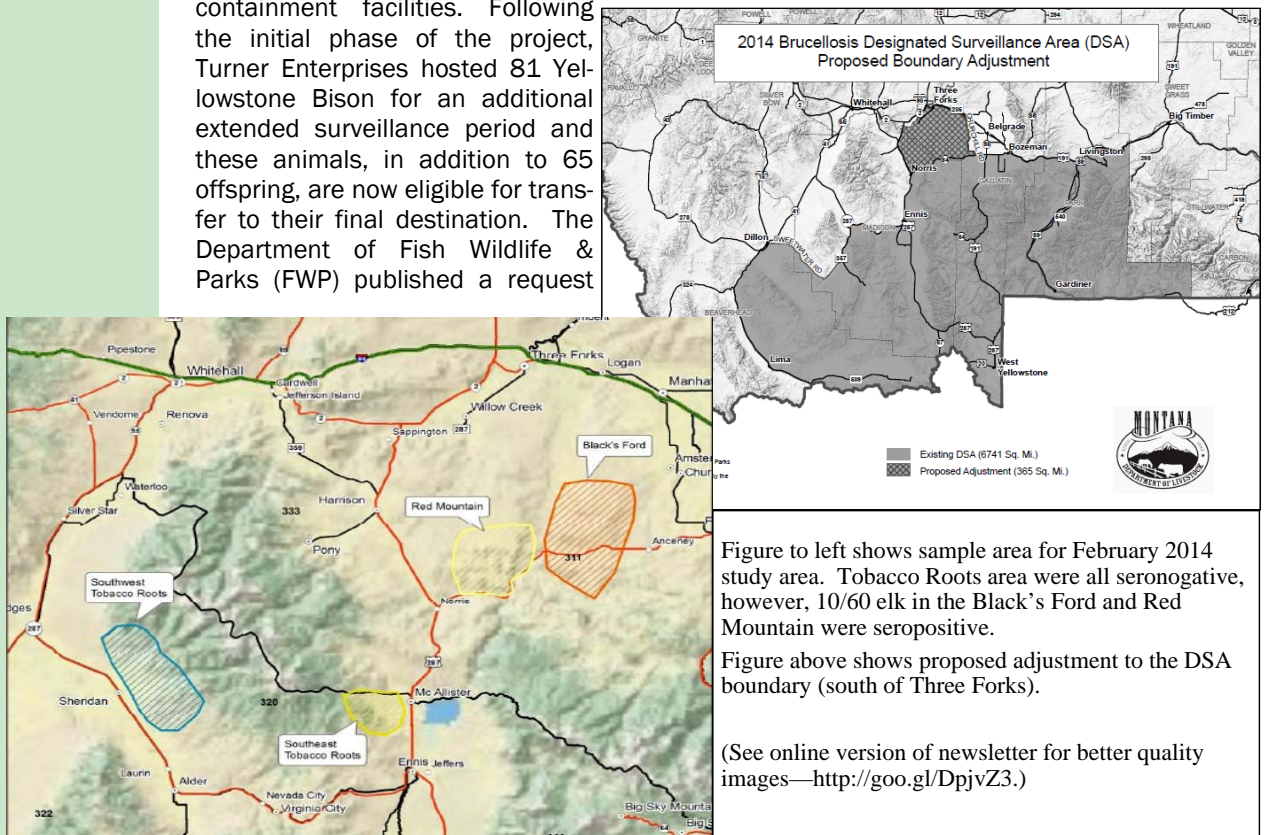


Figure to left shows sample area for February 2014 study area. Tobacco Roots area were all seronogative, however, 10/60 elk in the Black's Ford and Red Mountain were seropositive.

Figure above shows proposed adjustment to the DSA boundary (south of Three Forks).

(See online version of newsletter for better quality images—<http://goo.gl/DpjjvZ3>.)

## Animal Disease Traceability

**USDA TEST-TRACE EXERCISE:** DOL received a list of ten official tags that were distributed to Montana veterinarians between January 2008 and April 2010, and we're being asked where and when the tags were applied to animals. We found that for six of the ten tags, the veterinarians to whom the tags were issued do not have a ready means of finding tags. Unfortunately, thumbing through all health certificates over a 3 to 12-month period is not the basis for a successful trace.

It's not a surprise we're struggling with this challenge. The tags in this exercise not only predate the ADT rule, but also exceed the five-year records retention requirement established in the regulation. However, this is a great reminder that it's the veterinarian's responsibility to ensure that sufficient information is collected that will allow identification of the date, owner, and location for tags applied. Simply recording the tag range on a health certificate is not adequate; a log, either paper or electronic, that records date and owner/location for each tag range is ideal. We have seen spiral notebooks serve this purpose, but also have templates freely available.

**iCVI APP ON APPLE APP STORE:** The iCVI app is available in the App Store. The app has numerous features that should make issuing CVIs easier. Key features include:

- Import spreadsheets with official identification and other animal info.
- Populate consignor and consignee information from existing contacts.
- Saved certification statements.
- Resubmit, if corrections are needed.
- Completed documents automatically emailed to state of origin and any other individual you designate.
- CVI information will automatically be uploaded into our animal health database eliminating the need for data entry.

While you can set up a trial account and navigate in the app, submitting health certificates is currently limited to a handful of veterinarians who are beta-testing the app in Montana. The programming team wants to work out all the bugs prior to rollout to thousands of veterinarians nationwide sometime in the fall. ☒

By Tahnee Szymanski, DVM

## Porcine Epidemic Diarrhea Virus (PEDv)

PEDv has surprised many animal health experts in how rapidly it has spread since its introduction to the U.S. in April 2013. At last count, 30 states have reported cases with a typical finding of 100% mortality for several weeks of farrowing. Nationally, it's estimated that the disease has killed some seven million piglets with a subsequent decrease of market-ready hogs approaching 10%. Interestingly, PEDv is not the only novel coronavirus to emerge in the U.S. with Porcine Deltacoronavirus (PDCoV) also causing similar signs to PEDv.

In early June, USDA responded to this emerging threat by making the reporting of novel swine enteric coronavirus diseases (SECD) mandatory. Reporting of presumptive or confirmed positives is required by herd owners, producers, veterinarians, laboratory personnel, or anyone else with such knowledge. While the reporting of cases will be primarily performed by the diagnostic laboratory, the veterinarian will need to provide some additional information with the specimen submission including: a premises ID or location identifier (contact our office if you need this), date the sample was collected, and the type of unit being sampled (sow, nursery, finisher).

Additionally, USDA has made \$26.2M in funding available to combat SECD. Activities to be funded include \$3.9 million for development of vaccines, \$2.4 million to states to support management and control activities, \$500,000 to herd veterinarians to help with development and monitoring of herd management plans and sample collection, \$11.1 million in cost-share funding for producers of infected herds to support biosecurity practices, \$2.4 million to private sector veterinarians for diagnostic testing, and \$1.5 million to National Animal Health Laboratory Network diagnostic laboratories for genomic sequencing for newly positive herds.

Positive herds will need to follow a herd management plan and will receive assistance with testing and biosecurity costs. With this effort, USDA hopes to provide an incentive to testing and reporting which was lacking in previous surveillance efforts such as swine influenza testing.

Lots more information on SECD and the federal order can be found on the USDA and Pork Checkoff web sites. ☒ mz



Finishing hogs  
Source: Pork.org



Bison enrolled in the Bison Quarantine Feasibility Study (BQFS) at the containment facility.

## Montana Department of Livestock

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We're on the Web:  
[www.liv.mt.gov](http://www.liv.mt.gov)

## State Veterinarian Notes

*(State Veterinarian Notes continued from page 1)*

ence. All DOL rule changes are available for review on our Administrative Rule Notices page of our web site: <http://liv.mt.gov/public/arm.mcp.x>.

In other administrative updates, continuing education (CE) credits are now available for attending the Montana Veterinary Deputy Training. This training is held in conjunction with USDA "core" training and provides Montana veterinarians additional regulatory information to write health certificates, perform regulatory testing, and to act on DOL's behalf in cases of a disease incident. Category I accredited veterinarians are eligible for 3 CE hours, and Category II accredited veterinarians are eligible for 5 hours based on additional training materials provided at the training. ☒

mz

## Heartworm

*(Heartworm continued from page 1)*

In speaking with veterinarians from across the state, the trend has been to recommend chemoprophylaxis for all dogs. Initially veterinarians started with a three-month recommendation and now we are hearing of extended time frames (six-months.).

In the reported case, four of the fifteen dogs had been on preventative during the previous year. One had discontinued treatment in August and the remaining three discontinued treatment in October. Another theory of why a possible spike in heartworm cases is that mosquitos are surviving beyond the window that we are chemoprophylaxing.

As a gentle reminder, heartworm is a reportable disease in Montana. If you have cases, even back over the last year or two that have not been reported, we would be interested to hear about them. ☒

By Tahnee Szymanski, DVM

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