StockQuotes: Animal Health Newsletter

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Quarterly Newsletter from the Animal Health Division of the Montana Department of Livestock

Sep 2014

Volume 7, Issue 3

INSIDE THIS ISSUE

CALENDAR OF EVENTS:

Board of Livestock:
Sep 29-30, Helena

Deputy Veterinarian Training:
Sep 25, Helena

MVMA 2015 Winter Meeting:
January 30 - 31, Bozeman

Montana Legislature:
January 5th, 2015

Montana Stockgrowers Convention and Trade Show
Dec 11-13, Billings

State Veterinarian Notes

This newsletter contains a number of articles by subject matter experts on heartworm, genotyping and antibiotic sensitivity. Bear with us as we explore these topics in some depth.

The last StockQuote issue included a case report from a veterinary clinic in Flathead County about a number of heartworm positive cases. That article triggered some additional inquiries from veterinarians regarding prevalence of heartworm and risk to Montana resident cats and dogs. As it turns out, Dr. Greg Johnson (Montana State University) conducted a survey of Montana mosquitoes between 2005 and 2007, and I asked him to report on that study. Interestingly, based on a climatological assessment, the study singled out Lake County (adjacent to Flathead County) as an area west of the Continental Divide that could accumulate the necessary heat units for successful mosquito vector reproduction.

Also in the previous StockQuotes issue, Dr. Kammy Johnson (no relation), with USDA explained the current technology of genotyping *Brucella* isolates. In the second article on the topic, Dr. Johnson discusses how we can interpret not only the degree of similarity between *B. abortus* isolates (relatedness), but actually get some insight into which strain served as a source for the other.

Mary Ann Heagney, Clinical Microbiology Supervisor at the veterinary diagnostic laboratory provides some insight into antibiotic sensitivity testing.

Several administrative rules are being published for public comment including anthrax, tuberculosis testing of cervids, and elephant tuberculosis testing. You may have particular interest in the proposal to rescind the

(Continued on page 6)

WHAT'S NEW:

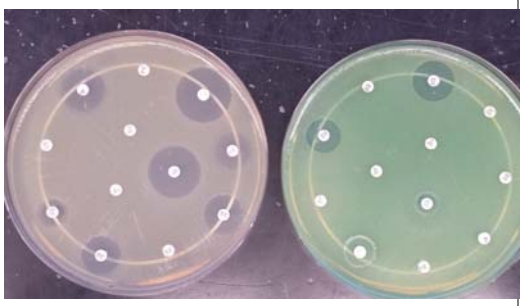
1. Administrative rule for brucellosis vaccination of imports just got published for public comment. http://goo.gl/mtCmGz (p4).
2. Update on Montana's trichomoniasis affected herds (p5).

Laboratory Corner: Antibiotic Sensitivity Testing

The Clinical Microbiology section of The Montana Veterinary Diagnostic Laboratory (MVDL) strives to provide accurate, timely, and clinically relevant results to practitioners.

One area of this service we are working to improve is reporting of antimicrobial susceptibility testing (AST) results. We intend to have antimicrobial drug panels that are specific for species, microorganism, and culture source (e.g. Bovine Respiratory Panel for *Mannheimia haemolytica*, *Pasteurella multocida*, and *Histophilus somni*). By testing only clinically relevant isolates, the practitioner can choose the best therapy and reduce inappropriate use of antibiotics.

MVDL uses the disk diffusion method to perform AST. This procedure uses antibiotic -impregnated disks that are placed on a plate inoculated with the bacterial isolate to be tested. A zone of inhibition of bacterial growth around the disk is measured to determine if an isolate is susceptible, intermediate or resistant (see Fig. 1 below).



The current (2013) versions of the Clinical and Laboratory Standards Institute (CLSI) veterinary standards are used to perform and interpret AST results. These standards provide the species-specific interpretive criteria for veterinary pathogens or human-derived interpretive criteria where veterinary interpretive criteria have not been established.

The CLSI standards also have guidelines regarding which organisms can be accurately tested by standardized disk diffusion AST methods. We have been adding comments

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Heartworm (Mosquito) Study

The study summary below has been provided by Dr. Greg Johnson, PhD, Montana State University, as a follow up to the heartworm column in the last issue. mz

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Image credit: http://www.petsafe.net/blog/wp-content/uploads/mosquito.jpg

Twenty-four species of mosquitoes have been identified as vectors of canine heartworm (HW) in North America with vector species varying geographically. While several of these species occur in Montana, we do not know which is the primary vector(s). Vector control and disease prediction and prevention can be greatly enhanced by knowing the mosquito species that transmits the parasite and identifying areas in the state that may be at greater risk for transmission.

In an attempt to better understand the local epidemiology of HW, a study was conducted to assay different mosquito species for the infective stage of the parasite and develop an infection risk map for the state based on temperature. Mosquitoes assayed for this study were trapped in 2005 and 2007 from sites east of the Continental Divide (Miles City, Hardin, Billings, Glasgow, Ft. Belknap, Helena and Townsend) and west of the Divide (Deer Lodge, Kalispell, Ronan, Missoula, Hamilton). We chose these two years because the summers were warmer than average and heat is critical for HW development. Therefore, selecting mosquitos from these summers might increase our chances of finding infected specimens. Species tested for HW included *Aedes vexans*, *Ae. trivitattus* and several *Anopheles* species; each has been reported to be a competent HW vector from other states.

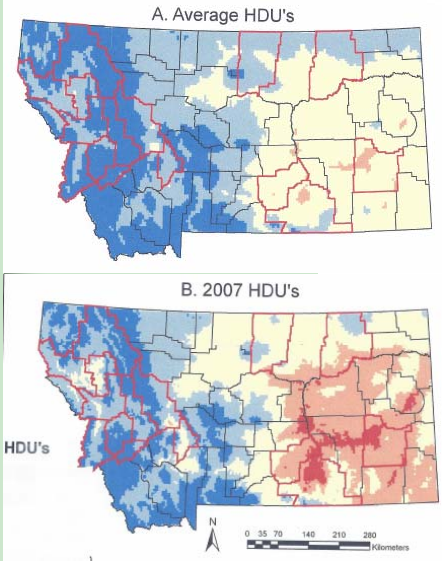


Figure 2: Maps showing difference in Heartworm Development Units between 2005 (top) and 2007 (below). Provided courtesy of Greg Johnson, PhD

For better quality images, please check the electronic version of this newsletter on the MDOL web site http://goo.gl/3jsf6O.

Over 60,000 mosquitoes were processed and tested for HW using RT-PCR assay. All mosquitoes were negative for HW DNA. This was not totally unexpected since the likelihood of detecting infected mosquitoes is very remote when local transmission rates are absent or relatively low. Despite not detecting HW, we know suitable vectors are present based on previous transmission records to native dogs with no travel history out of the state (Knapp et al. 1993. J. Parasit. 79:618-620, Bowman et al. 2007. Vet Therapeu. 8: 293-304).

We did not test *Culex tarsalis*, a species that was recently reported as a HW vector in northern California (Huang et al. 2013 JME. 50:1315-1323). That study included assays of over 36,000 mosquitoes and found this and six other species positive for HW. *C. tarsalis*, associated with irrigated agricultural and wetlands, is common in Montana and is more abundant in counties east of the Continental Divide. Over the years we have detected a surge in abundance of this species, as well as others, in many eastern and north central trap sites in the state. It also occurs west of the Divide but densities are comparatively lower. In future HW detection studies this would be a species to focus on along with some of the other previously tested species.

A second aspect of our study was to develop an infection risk map based on average daily temperatures showing where heat units accumulate in the state for development of HW in the vector. A minimum of 130 heat units, using 14°C as the threshold, is required for development to the infective stage within the mosquito. Based on 30 year temperature averages, several foci exist in Yellowstone and Big Horn counties and counties along the Yellowstone River where temperatures often exceed the 130 heat unit requirement. These areas would be considered to be at a higher risk for transmission. The remainder of the state, including Lake County, was typically below the minimum heat units necessary for parasite development and at negligible risk for HW transmission.

However, the potential for new or even increased transmission in areas does exist when temperatures increase. Areas acquiring 130 or more heat units greatly expanded in 2007 compared to 2005 (Fig 2). This included a large portion of eastern Montana and sites west of the Divide in Lake, Missoula and Ravalli counties. Unfortunately, predicting HW transmission is difficult due to the complex interactions of the biological components (infected hosts, density of hosts, longevity of putative vectors, etc.) and environmental factors (precipitation, heat, etc.), but being cognizant of heat unit accumulations may be a useful tool helping to determine the potential for HW transmission. ¤

By: Greg Johnson, Montana State University

Grant Hokit and Sam Alvey, Carroll College

Genotyping 102

In the last issue, we examined the methodology behind Whole Genome Sequencing (WGS) for Brucella abortus and began to see the results of this new methodology. This issue will delve deeper into the output of this method and show how bacterial single nucleotide substitutions – or SNPs - are informing current disease control and eradication efforts.

Two types of output result from WGS – trees (low and high resolution) and tables. Both tables and trees show how field isolates compare to a known reference strain. Tables compare isolate differences at the individual nucleotide level while trees are a graphic representation of relatedness between isolates in the analysis.

Low resolution (LR) trees (see Fig. 3 to the right) graphically depict the relatedness between all isolates in the database. LR trees are "30,000-foot-view" family trees that show major groups or 'clades' of isolates; think of a clade as a nationality or clan. When looking at a LR tree, remember that the length of the branches are not significant and the shape of the tree can vary. Using LR trees, it is clear that there are four distinct ancestral lines or clades of B. abortus circulating in the GYA. Furthermore, each clade within the GYA is circulating within a distinct geospatial area.

Isolates within a clade undergo further highly specific analysis to show individual level relatedness. The SNP table illustrates how detailed SNP information is shown and closely related isolates are grouped. High resolution (HR) trees are the graphic representation of this same information (see Fig. 4 bottom right). When looking at a HR tree, the angles are irrelevant, however, the length of the arms reflects the number of SNPS between isolates. Figure 4 shows the relationship between a SNP table and a HR tree for five select isolates originating from MT animals. The top isolate in the SNP table is, in genetic terms, "older" or ancestral to those below it, as evidenced by having eight SNPs different from the reference isolate (SNPS are shown as colored nucleotides). All isolates below it are highly related since they share it's genotype but have additional SNPs that have occurred over biological time.

It is worth noting that four isolates originated from domestic cattle or bison and one from wildlife over the span of four years. While WGS evidence cannot prove the directionality of the spread of infection, the high level of relatedness between isolates suggests that a single, pre-ancestoral) isolate has been circulating among these populations during that time period. Unfortunately, that pre-ancestoral isolate is missing in the database, but like the long lost uncle, we know it is there!

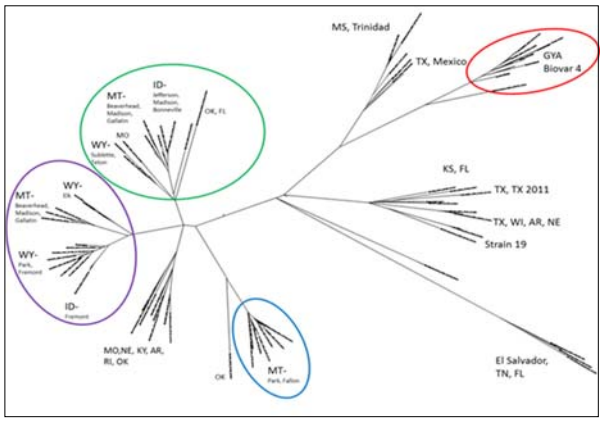
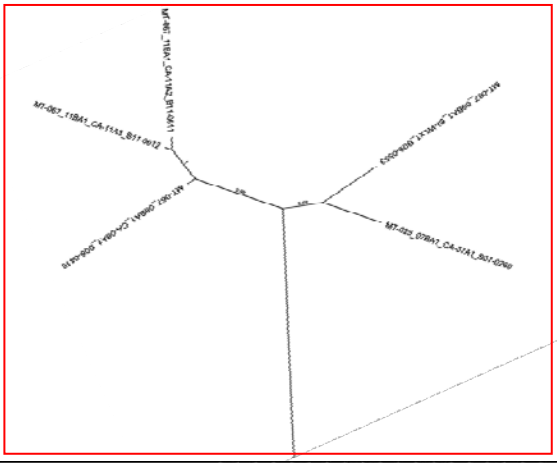


Figure 3. Low resolution tree of *B. abortus* isolates (historical data).

Using the frequency of SNPs in the bacterial genome, the geospatial clustering of clades, the genetic relationship between individual isolates/ infections within a geospatial area, and traditional epidemiology allows for a detailed look at how this bacteria is behaving on the landscape and insight on potential methods of disease eradication and control. ¤

By Kammy Johnson, DVM, USDA-APHIS-VS

Figure 4: High resolution tree and SNP table of five MT-origin *B. abortus* isolates. A longer branch length indicates greater differences in the genome of the *B. abortus* isolate.



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reference_call	T	G	C	A	G	T	C	T	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_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

Administrative Rules Proposals

I appreciate the time that many of you take to familiarize yourself with rule proposals. While the official filings are available on our website and provide the most comprehensive information, summaries of proposed rules that will be published in the next several weeks are below:

BRUCELLOSIS VACCINATION OF IMPORTS: Following an abortion storm associated with adult brucellosis vaccinated cattle imported into Montana, I requested that the Board of Livestock allow us to review the vaccination policy on imports. Based on this review, we are proposing to remove the brucellosis vaccination requirements for states that have been brucellosis free for 10 or more years. This recommendation is based on the fact that the majority of U.S. states have been free of cattle brucellosis (*Brucella abortus*) for many years and imports from those states do not pose an appreciable risk of brucellosis. Therefore, we are proposing to amend administrative rules 32.3.221 and 32.3.212A which opened for public comment on September 18.

Of note, Montana unvaccinated calves and adult cattle are accepted for importation by many states. As we request other states to use a risk based approach for import requirements of Montana's cattle, we should administer the same standard to Montana imports.

While not entered as part of the official comment record into this rulemaking, it seems that the comments we received in 2010 on a proposed statewide OCV requirement might apply. Those comments which predominantly argued against a statewide vaccination requirement emphasized: a) incomplete efficacy of the vaccine (indeed, nearly all reactors in Montana have been brucellosis vaccinates); b) additional workload and financial burden for uncertain benefit; and c) the risk of brucellosis is limited to the range of brucellosis infected wildlife.

Administrative rule 32.3.436 (Vaccination within the Counties in which the DSA is Located) is not proposed to change. 32.3.436 requires that all female cattle and domestic bison 4 months of age or older within Beaverhead, Gallatin, Madison and Park Counties must be official brucellosis vaccinates by January 1 of every calendar year.

ANTHRAX: DOL's regulations on anthrax are significantly outdated. The prescribed disinfectant (quick lime) can actually increase spore formation creating a greater risk in the future. Also, the rule as written, quarantines animals for an extended period and limits the use of vaccination to the area immediately affected. We are proposing to address the shortcomings of rules 32.3.1001 and 32.3.1002. These proposals will be open for comment on October 9.

TUBERCULOSIS TESTING OF CERVIDS: We are proposing to revise administrative rule 32.3.221 to simplify the TB testing requirement to one standard; one negative approved tuberculosis single cervical test on all sexually intact animals within 90 days prior to importation and part of a whole herd test within the last 12 months. While we typically base import requirements on disease status (TB-Free, etc..) of the source state, we do not anticipate that state classifications for Cervid TB are going to change from Modified Accredited in the foreseeable future. Therefore, the proposed rule lists just one testing standard for import.

In rule 32.3.602A (Change of Ownership Test), we are clarifying that the requirement for tuberculosis testing applies to intrastate movement and change of ownership. This intent is supported by other rules and is consistent with DOL's current interpretation of the rule. These rules will be open for public comment on October 9.

ELEPHANT TUBERCULOSIS TESTING FOR IMPORTS: This rule is primarily in place to ensure that elephants coming into Montana for exhibition events don't present a risk of tuberculosis to other animals or people. The recommended screening test, the Elephant TB Stat Pak, is no longer manufactured. The proposed language eliminates the Stat-Pack and does not replace the requirement with a specific test to prevent the rule from being quickly outdated.

It's important to keep this rule current because of numerous documented cases of TB in circus elephants, the difficulty in treating these cases, and the potential risk to public health and other animals. Rule 32.3.227 Elephants, will be open for public comment on October 9. ¤

By mz and Tahnee Szymanski, DVM

Trichomoniasis

UNIFORMITY OF TRICHOMONIASIS REGULATIONS: As of July 2014, 27 states have interstate trichomoniasis (trich) regulations. Unfortunately, there is too much variability in states' requirements which creates delays in shipment of bulls, unnecessary testing, and other costs.

Areas where states' regulations differ generally fall into four categories:

- Accepted test — culture or polymerase chain reaction (PCR)
- Validity of negative test — usually from 30 to 60 days
- Whether pooling of samples is accepted
- Age of bulls that can be exempted from testing — usually from 12 to 24 months of age

Because the bull-testing protocol depends on the state of destination, bulls can't be tested until after sale — when the state of destination becomes known. Bulls must be held for days after the sale while samples are collected and processed by the laboratory, preventing animals from leaving with buyers post-sale, which increases costs on both sides.

Fundamentally, regulations have no standard because when rules were created, there was no national consensus. Absent a technical advisory group or federal standard, states wrote regulations based on their experience with trich and input from state stockgrower groups.

Fortunately, there is growing interest in making trich rules for interstate commerce more consistent. To that end, DOL administered a survey to state animal health officials looking for consensus in the 4 main categories with some encouraging results. There is an 89% or higher agreement on the type of acceptable test (single PCR), and age of virgin bulls exempted from testing (18 months of age). Agreement on duration of test negative (60 days) is at 82% and agreement on accepting results from lab-pooled samples is 78%. The survey will be repeated in 2015.

Full survey results are available at https://www.surveymonkey.com/results/SM-3LH6FKZ8/.

MONTANA CASE UPDATE: There has not been a diagnosis of trichomoniasis in a Montana herd since January of 2013. This herd was tested as an adjacent to a positive herd diagnosed in December of 2012.

Currently, Montana has four herds that remain classified as trich positive.

1. A herd in Glacier County that has completed none of the requirements of a trich positive herd, including neighbor notification. This herd falls under Blackfeet tribal jurisdiction.
2. The January 2013 herd which has assurance test pending; enforcement actions have been initiated.
3. Another herd in Yellowstone County that has also failed to complete the requirements placed on a trich positive herd.
4. A herd currently located in Lewis and Clark County that is part of an ongoing legal dispute over ownership. This herd is awaiting a post breeding test on its entire bull herd.

These herds remain flagged in our brand system to ensure that all animals sold from these herds are destined for slaughter only. Of interest, we recently discovered that one of the trich herds listed above has been running in common with other herds and selling cattle under the brand of a family member. We have since flagged all of these brands and will require a negative test of all bulls associated with these herds in order to clear them from market restrictions.

Despite these few "problem" herds, MDOL is confident that the incidence of trich in Montana remains low. Test numbers remain strong (see Fig 6). ¤

By mz and Tahnee Szymanski, DVM

Figure 5: Trichomoniasis tags changed to **YELLOW** on Sept 1st.

Color schedule is as follows:
Sep 1, 2014 - Tag color changed to Yellow
Sep 1, 2015 - Tag color changes to **Green**
Sep 1, 2016 - Tag color changes to **White**
Sep 1, 2017 - Tag color changes to **Orange**
Sep 1, 2018 - Tag color changes to **Blue**
Sep 1, 2019 - Tag color repeats (**Yellow**)

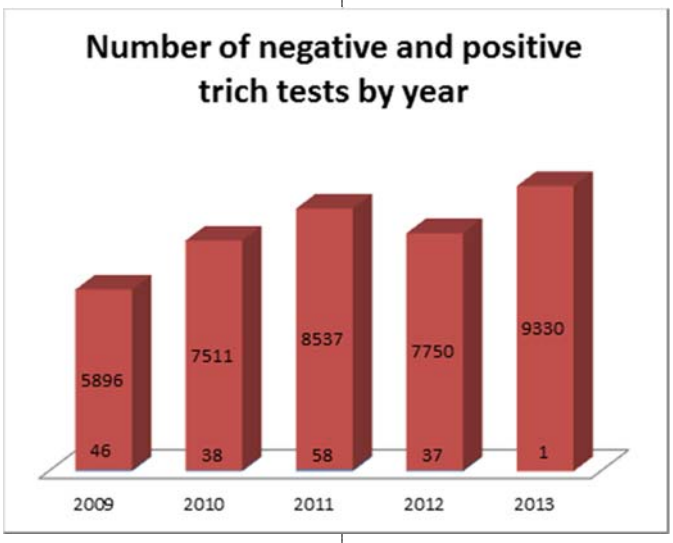

Figure 6: Trichomoniasis tests by year 2009-2013. Positive tests by year are 46 (2009), 38 (2010), 58 (2011), 37 (2012), and 1 (2013).

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State Veterinarian Notes

(State Veterinarian Notes continued from page 1)

requirement for calfhood vaccination on imported female cattle from brucellosis-free states. These are covered in some depth in the administrative rules section.

In the trichomoniasis column, Dr. Szymanski provides an update on the status of current cases, and I discuss some recent efforts at trying to bring some consistency to interstate requirements.

This issue of the Montana One Health provides information on the public health impacts of consumption of unpasteurized ('raw') milk products. With the legislative session right around the corner, we're expecting that this topic will receive more attention.

If you don't get our email updates (but would like to) please be sure to contact our office and provide a valid email address. Recent email notifications included information about anthrax field test kits, cases of West Nile virus, the DOL budget and numerous other topics. ¤ mz

Antibiotic Sensitivity Testing

(Antibiotic Sensitivity Testing continued from page 1)

to reports in these cases. One example of this is reporting a susceptibility for a *Corynebacterium* species; there are no standardized disk diffusion methods or interpretations, so that information is provided. There are also organisms that should not have AST performed due to predictable results; e.g. *Trueperella pyogenes* (formerly *Arcanobacterium pyogenes*) is predictably susceptible to penicillin and macrolides, so AST is not necessary.

The use of standardized testing methods and reporting of susceptibility test data have become critically important in understanding resistance development in veterinary pathogens and the development of judicious use guidelines for veterinary antimicrobial agents. The MVDL is striving to meet these goals to provide the best service to our clients. ¤

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