EQUINE DISEASE QUARTERLY

FUNDED BY UNDERWRITERS AT LLOYD'S, LONDON

OCTOBER 2016
Volume 25, Number 4

COMMENTARY

IN THIS ISSUE

In this issue of the Equine Disease Quarterly, Dr. Emma Adam discusses the new genomics tools available to study horse diseases. Her authorship of this article is particularly noteworthy since Emma initially trained as a veterinary surgeon before returning to university to complete a Ph.D. studying joint diseases in horses. She discovered that genomic tools were an effective way to address problems that were not resolvable using earlier technologies. Her article presents the "nuts and bolts" of horse genomics. As a point of reference, in 1990 we had only characterized 50-100 genes for the horse and confirmed the chromosome location for a mere seven of them. Fast forward to 2009, and the whole genome sequence for the horse had been determined. The immediate application of the sequence data at the time was to identify DNA mutations responsible for well-known diseases of the horse. However, that was only the beginning. There was a drive to find out how genes function.

Everything that we do to a horse turns genes on or turns them off. If genes are defective, it can result in development of disease. Genes are also important for performance. Some genes have variants that affect such things as type of gait, optimal racing distance to reach performance potential, and behavior. Many genes interact with management practices such that horses may be more reactive or, alternatively, less responsive to certain feeding programs, training regimens, or vaccinations. Breeders and trainers attempt to optimize management. These observations clearly suggest that genomics information has a place both in veterinary practice and in the stable-yard.

Emma also has been among the scientists developing tools to investigate genes, their expression, and their impact on horses. Before genomics, we basically fed, trained, or treated horses then observed them to assess what the clinical or phenotypic effects might be. Emma's studies pioneered another approach. Her studies entailed comparing gene expression in joints and other collagenous tissues at different stages of life, including several stages of embryonic development, and then assessing which genes had an impact on healthy growth of that tissue. Understanding the genes that contribute to tissue development and repair should lead to development of veterinary therapeutics that benefit the health and welfare of the horse and rider.

Emma is not alone in pioneering the use of genomics for research on horses. A quick survey of veterinary publications reveals scientists using genomics to study reproduction, lameness, respiratory diseases, infectious diseases, immunology, and more. We do not need to become molecular geneticists to enjoy horses any more than we need to become mechanics to drive a car. But we need to know, appreciate and encourage development of these new approaches. Watch that space!

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LLOYD'S

INTERNATIONAL

Second Quarter 2016*

The International Collating Centre, Newmarket, United Kingdom, and other sources reported the following disease outbreaks.

In early April, a case of African horse sickness was reported in the Surveillance Zone, Western Cape Province, Republic of South Africa. A total of 20 additional cases of infection with serotype 1 virus were subsequently identified on seven other premises.

Equine influenza was reported by the UK and the USA. One outbreak involving a limited number of cases was recorded in the UK. The disease is endemic in the USA; it was confirmed in six states.

Equine herpesvirus 1 and 4 (EHV-1, -4) related diseases were reported by France, Germany, Ireland, Japan, Korea, Switzerland, the UK, and the USA. Respiratory disease was diagnosed in France (seven outbreaks, all isolated cases), Germany (nine cases involving eight premises), Ireland (four cases), and the USA (activity recorded in various states). Abortion due to EHV-1 was confirmed in France (two outbreaks), Germany (five cases), Japan (single cases on three premises), South Korea (one case), Switzerland (two cases in neonatal foals, one co-infected with *Actinobacillus equuli*), the UK (three outbreaks), and the USA (three cases). EHV-1 neurologic disease was reported by France (two outbreaks) and the USA (four outbreaks). Neuropathogenic or non-neuropathogenic virus strains were involved. EHV-4 respiratory disease was recorded by France (14 outbreaks), Germany (nine cases on six premises), and Switzerland (one outbreak involving co-infection with EHV-1 and -4).

Strangles was reported by France (eight outbreaks), Germany (10 cases involving eight premises), Switzerland (three affected premises), and the USA in which the disease was considered endemic, with 57 affected premises recorded in 17 states, 10 with multiple outbreaks.

The USA identified two Warmblood stallion carriers of equine arteritis virus.

A limited number of cases of EHV-2 and/or -5 infection also were recorded in the USA.

Equine infectious anemia was reported by Canada and the USA. Canada confirmed a total of 15 cases, one on a premises in British Columbia and 14 divided between four premises in Saskatchewan, two linked epidemiologically. One case was diagnosed in a non-racing horse in Colorado, USA.

France (endemic), Spain (endemic), and the USA reported cases of equine piroplasmosis. There was an increase in case numbers in the USA; all were *Theileria equi* positive Quarter horse racehorses, the majority in Texas, with some involved in bush track racing.

Salmonellosis was recorded in Germany (one case) and the USA (seven outbreaks involving Group B *Salmonella* spp. and one Group C1 *Salmonella* spp.). Outbreaks of rotavirus infection were confirmed in France (13 outbreaks). One involved co-infection with rotavirus and coronavirus. The USA reported outbreaks of clostridial enteritis; *C. difficile* was implicated in two outbreaks and *C. perfringens* in seven others. Isolated cases of coccidiosis and *Mycobacterium avium* were recorded by Switzerland and Singapore, respectively. Contagious equine metritis was reported by Germany, with 20 cases confirmed on 12 premises, the majority in stallions. Icelandic, Warmblood, and Coldblood breeds were involved. The USA diagnosed five cases of equine coital exanthema (EHV-3) and one case of nocardioform placentitis (*Amycolatopsis* spp.).

A total of 18 cases of Eastern Equine Encephalomyelitis were recorded in the USA, with the majority identified in Florida (11) and South Carolina (5).

West Nile Encephalitis reappeared in the second quarter, with single cases confirmed in California and Florida.

The USA reported one case of equine rabies in Arizona.

Switzerland recorded two cases of ehrlichiosis on separate premises.

**First Quarter Report from Australia*

Equine Disease Quarterly

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Pandora's Box – Equine Genomics

The genetics of horses has been of great interest to humans for millennia. While we may not have used that term specifically, the recording of equine parentage, births, and deaths was a practice that pre-dated the compulsory recording of human births and deaths in Great Britain by over a century. The term "genomics" covers all aspects of genes, including their structure and function, not just the science of heredity.

The genome is all of the DNA, divided up and packaged as chromosomes, in each cell. The DNA molecule, the unit of heredity, is made up of four nucleotide bases, guanine (G), cytosine (C), adenine (A), and thymine (T) in a sequence. The equine genome consists of ~2,700 million base pairs, which is similar in size to the human genome.

Less than 3% of the genome actually codes for proteins. The remaining 97% was formerly termed "junk DNA" but we now know it orchestrates the use of the entire genome, as regulatory elements. Individuals have differences in the sequences of the nucleotides. This is pretty obvious - we have greys and bays but they are still horses. However, not all of the sequence differences are that obvious, and sequence differences can be whole sections of sequence or just single nucleotides. A Single Nucleotide Polymorphism (SNP, usually pronounced "snip") is a single nucleotide in a sequence that differs between individuals at a low population frequency.

Approximately 10 million SNPs have been found in the collective equine genome. It is important to remember that SNPs are not necessarily mutations that have any effect on the organism. Indeed, SNPs are less likely to be found in the protein coding genes of the genome as these areas have been heavily selected for functionality by evolution. Any change in DNA sequence that did not benefit the animal would reduce its chances of survival in the gene pool.

Science uses selected SNPs as a crude road map of the genome. By way of analogy, a set of directions are like a SNP map. If I gave you some directions, they might be: "Turn right at the pub, then take the second left after the church." The pub and the church have no actual bearing on the destination, they are just guiding landmarks, and so it is with SNPs. SNPs that sit close together on a chromosome are likely to be inherited together. Using commercial SNP arrays ("SNP chips"), molecular biology techniques can rapidly hone in on a region of the genome that is different between horses in terms of its SNP frequency.

SNP analysis is an immensely useful tool to narrow down the search for areas of the genome harboring genetic traits of interest, whether they be a trait relating to disease or a desired trait. Studies using this approach are called Genome Wide Association Studies or GWAS (pronounced 'gee-waahs').

The SNP-GWAS approach has helped identify regions of interest in the genome in diseases such as Lavender Foal Syndrome, Polysaccharide Storage Myopathy, recurrent laryngeal neuropathy, Foal Immunodeficiency Syndrome in the Fell and Dales pony, osteochondrosis dissecans (OCD), guttural pouch tympany in Arabians and German warmbloods, recurrent uveitis in German warmbloods, insect bite hypersensitivity, and hydrocephalus in Friesians.

We have only just started to uncover the wealth of information the equine genome holds. Continued efforts for funding and research will yield more mind-blowing results than our imagination can fathom. Watch this space!

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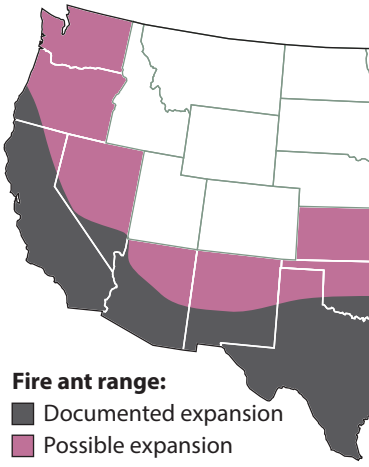
Figure 1. Fire ant colonies in the U.S.



Source: http://www.ars.usda.gov/Research/

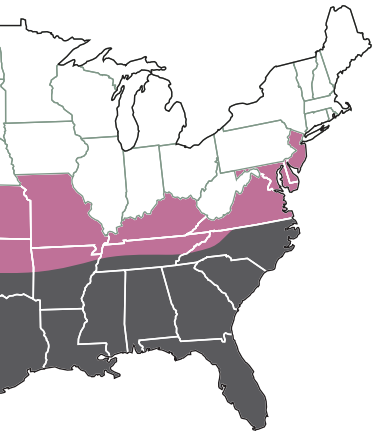
NATIONAL

Fire Ant Surveillance for Horse Farms

The red imported fire ant occurs in parts or all of Alabama, Arkansas, California, Florida, Georgia, Louisiana, Mississippi, New Mexico, North Carolina, Oklahoma, Puerto Rico, South Carolina, Tennessee, Texas, and Virginia. Occasionally, it has been found in Kentucky, Maryland, and Missouri (Figure 1). Fire ants like to establish colonies in open sunny fields and pastures. Soil moisture and winter temperatures round out the major environmental factors that limit the spread

of this invasive insect. Changes in climate, along with the adaptability of the insect point to a continued gradual expansion of its boundaries.

The impact of the fire ant extends beyond the pain of its legendary sting. No significant adverse effects to the health of foals or mature horses have been reported in states in which fire ants are widely established. In addition to causing injury to workers, animals, and wildlife, this small insect affects pasture maintenance, hay production, damages equipment, and increases costs. Horse-farm managers in fire ant-infested areas have adjusted their management practices and developed strategies to live with this pest. The gradual northward and westward expansion of the fire ant's range exposes more farm managers and stable and pleasure horse owners to this important pest. Those living along the expansion front should become familiar with some of the basics of fire ants and watch for ant activity that seems out of the ordinary.



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The familiar mound is the ant's hallmark, but there is one major difference when it comes to fire ants: Their mound is the typical pile of loose, fine soil but there is no central opening. Instead, fire ants enter and leave their colonies through underground tunnels that radiate from the mound. Mound heights range from a few inches in mowed areas to 18 inches in undisturbed areas. Repair of a fire ant mound collapsed by a heavy rain results in a loose, fluffy pile of soil a few days later.

Fire ants look like typical ants. They are small but vary from 1/8- to 1/4-inch in length. The head, thorax, and legs are red to brown with a black abdomen. Positive identification of fire ants requires collecting approximately a dozen specimens in rubbing alcohol and taking them to your local Cooperative Extension Office. This must be done carefully. Disturbing the mound usually prompts numerous ants to pour out and climb up any vertical surface to sting the intruder. Other ant species scurry about when the colony is disrupted, working to protect the queen and move their brood to a safe place.

Collect the ants carefully to prevent being stung. Dust baby powder on dishwashing gloves and wear them because the ants cannot crawl up dusted surfaces. Stay as far away from the mound as possible during collection and watch for ants crawling on your shoes.

Follow up a positive identification of fire ants with a careful examination of the property in spring or late fall to determine the number and location of active mounds. Fire ants are managed by careful application of baits or mound drenches of insecticides labeled for fire-ant control. Take advantage of the excellent information available on fire-ant management.

For more information, see *Identifying Fire Ants,* http://articles.extension.org/pages/11278/identifying-fire-ants.

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The Asymptomatic Carrier Stallion: Critical Role in Venereal Disease Transmission

A range of venereally transmissible agents—viral, bacterial, and protozoal—have long been known to establish persistence or the carrier state in stallions, mares, or both. Some of these agents (e.g. *Pseudomonas aeruginosa*, certain capsule types of *Klebsiella pneumoniae* and *Streptococcus zooepidemicus*) are commonplace in most domesticated horse populations. Others such as equine herpesvirus 3, equine arteritis virus, *Taylorella equigenitalis*, or *T. asinigenitalis* are less frequently encountered. Of additional significance is *Trypanosoma equiperdum*, the causal agent of dourine, which though rarely reported nowadays, is reputedly still present in certain regions/countries of the world.

Even though some but not all of the foregoing agents can establish persistent infection in both the stallion and the mare, it is the carrier stallion that plays a more important role in the epidemiology of the respective infections. Not only has it the potential to disseminate a particular infectious agent among the mares to which it is bred, but of even greater long-term significance, it ensures the transfer of infection from one breeding season to the next.

While some of these agents, such as equine arteritis virus and *T. equigenitalis*, can be transmitted either through natural service or artificial insemination, the risk of more widespread transmission is much greater through the practice of artificial insemination with fresh-chilled or frozen semen from a carrier stallion. This was borne out in the course of the 2006 equine viral arteritis disease event in the USA, when fresh-chilled semen from a well-known Quarter horse stallion

in high commercial demand was responsible for spread of the virus to breeding stock in 18 states and two provinces in Canada, all within a two- to three-week period. This resulted in outbreaks of equine viral arteritis, abortion in naïve pregnant mares, and establishment of the carrier state in a variable number of exposed stallions.

It must be emphasized that stallions that continue to harbor equine arteritis virus, equine herpesvirus 3 or *T. equigenitalis* are asymptomatic or clinically inapparent carriers. With the exception of infection with *T. equiperdum*, there is no means of knowing whether a stallion is a carrier of a particular venereal pathogen or not without subjecting it to appropriate testing protocols for whatever the agent under consideration might be. Regardless of what venereal infection is being screened for, however, it is critically important that such testing is carried out by a reputable veterinary diagnostic laboratory with an established record of competency and experience in testing for that infection. The reliability of laboratory testing is crucially important to the success of any prevention and control program especially in the case of equine viral arteritis and contagious equine metritis.

A further confounding factor when dealing with stallions that are asymptomatic carriers of equine arteritis virus or *T. equigenitalis* is the fact that the majority of naïve mares to which they are bred may subsequently exhibit minimal, if any, clinical evidence of infection. This leaves the breeder/mare owner unaware that transmission of infection has occurred and that the stallion in question is a carrier of either of these two venereal pathogens. This could have significant consequences in the case of equine arteritis virus should such an acutely infected mare be pastured with naïve pregnant mares. The 2008-2010 CEM event in the USA illustrated how easily a stallion that was a carrier of *T. equigenitalis* could escape detection at time of importation and ultimately be responsible for the very costly event that first came to light in 2008.

Past and recent experiences underscore the importance of screening breeding stallions regardless of breed, for presence of the carrier state. This applies especially to equine arteritis virus. Moreover, the responsibility for ensuring the safety of breeding stallion populations ultimately resides with the equine industry.

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KENTUCKY

The Importance of Cleaning to Disinfection

Cleaning and disinfecting stalls is critically important for biosecurity, especially in controlling disease outbreaks. However, much misinformation exists.

The average 1,000-pound horse produces 50 pounds of manure and urine per day. Add on to that other body fluids that potentially contain pathogens (nasal discharges, abscess material, blood, etc.), and significant organic load exists in the average horse stall. Any surface that needs to be disinfected (treated with chemicals in order to kill pathogens) must be cleaned of dirt and organic material first.

Cleaning a stall takes detergent and manual labor. Power washers should not be used to avoid aerosolizing pathogens. Despite advertising claims, no "one step" product exists that can be sprayed on a dirty stall and effectively kill pathogens. Surfaces must be scrubbed with a detergent or cleaning agent to loosen and remove as much organic matter as possible.

Detergents are cleaning agents that emulsify (loosen) organic matter without forming a "soap scum" residue. A detergent should be used to scrub stall surfaces followed by rinsing to physically remove dirt and organic matter. Only after surfaces have been cleaned should they be sprayed with a disinfectant.

Studies have shown that over 90% of bacteria are removed from surfaces that are thoroughly cleaned first. Considering that equine herpesviruses, influenza viruses, and equine arteritis virus are lipid-enveloped, cleaning surfaces with detergent will disrupt this envelope, helping to render these viruses inactive.

While bleach is an effective disinfectant on "hard, non-porous, previously cleaned surfaces," horse stalls on farms are rarely constructed of

Equine Disease Quarterly Newsletter

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such materials. Bleach is also rapidly inactivated by organic matter.

Disinfectant labels state: "It is a violation of Federal law to use this product in a manner inconsistent with its labeling." Users should understand and follow label instructions and call the manufacturer with any specific questions. If the label states "dilute ½ ounce of disinfectant concentrate in one gallon of water," use that dilution. Increasing the amount of chemical assuming it will overcome a dirty surface is a waste of time and money and could pose health hazards to people and animals.

Never mix different disinfectants together. For example, bleach combined with ammonia or strong oxiders can produce lethal gas and dangerous chemical compounds. Every approved disinfectant in the USA has a Safety Data Sheet (previously known as Material Safety Data Sheet) which is available from the manufacturer and contains valuable information.

The statement "Proven effective against the following organisms," followed by a long list of pathogens, is on many disinfectant labels. However, in the fine print is how this list was generated. Most disinfectants have been tested in the presence of 5% serum as the "organic" load. A feces-stained stall wall has an organic load much higher than 5% serum, which is why cleaning is critical to the effectiveness of any disinfectant.

Excellent infection control and disinfectant information is available at www.cfsph.iastate.edu and at www.aaep.org.

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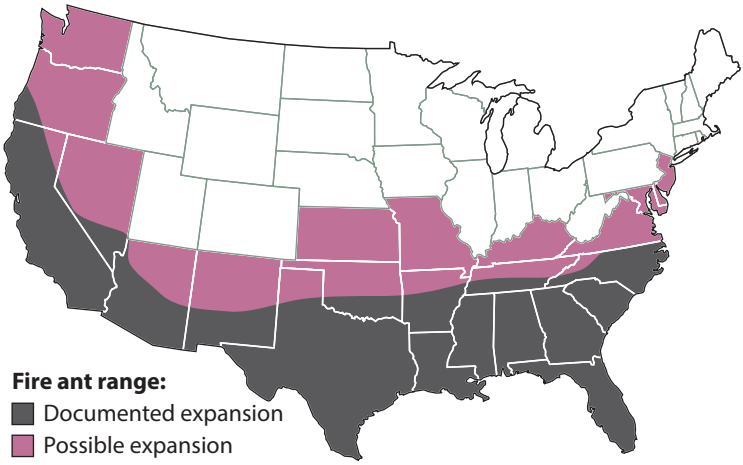
Figure 1. Fire ant colonies in the U.S.



Source: http://www.ars.usda.gov/Research/docs.htm?docid=9165&pf=1&cg_id=0