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EQUINE DISEASE QUARTERLY

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RESEARCH SPOTLIGHT

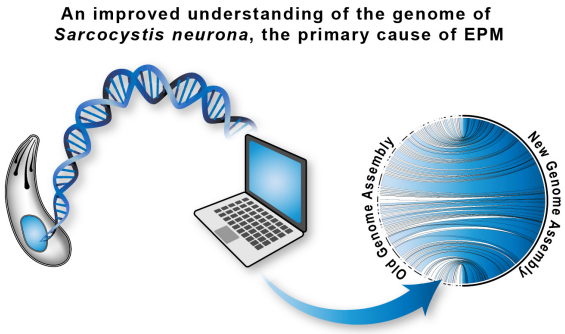
An improved understanding of the genome of *Sarcocystis neurona*, the primary cause of EPM

Our lab is working to solve equine protozoal myeloencephalitis (EPM), one of the most commonly diagnosed neurologic diseases in horses in North and South America. Annual direct costs associated with the diagnosis and treatment of EPM represents a large economic burden to the equine industry and is estimated to be between $55.4 million and $110.8 million in the United States alone. This does not account for the additional economic impact due to lost production, decreased performance and the cost of care during recovery. Even after successful veterinary intervention, full recovery from the disease can be difficult due to lasting damage to tissues of the central nervous system.

EPM is caused primarily by Sarcocystis neurona, a single-celled parasite. Normally, Sarcocystis has a two-host lifecycle where it spends most of its time in small mammals such as skunks, racoons and nine-banded armadillos. After asexual replication, parasites will form dormant cysts (sarcocysts) in the muscle tissue of this animal, which are later ingested through scavenging by the opossum definitive host.

In the opossum, the parasites excyst in the intestines, where they sexually replicate to produce environmentally-stable infective sporocysts that are passed along with feces into the environment. Interestingly, the natural lifecycle of S. neurona does not include the horse. Horses become accidental hosts when they ingest feed or water contaminated with opossum feces containing these sporocysts. In the horse, the parasites excyst in the gut and migrate to the brain and/or spinal cord where they cause local inflammation and tissue damage. Depending on the location of this inflammation, clinical signs can vary to include slight incoordination, asymmetric muscle atrophy, partial facial paralysis, difficulty swallowing, ataxia, recumbence, seizures and/or death.

Of 5,250 horses sampled in 2013 from 18 states across the United States, seroprevalence of antibodies against S. neurona was 78% indicating that horses are commonly infected by this parasite. A study by the National Animal Health Monitoring System in 2001 reported the annual occurrence of EPM to be approximately 0.14% in the US, with case fatality rate at 4.7%. The large discrepancy between the seroprevalence of antibodies to S. neurona and the low occurrence of EPM is puzzling and suggests that disease pathogenesis is complex.



Initially revolutionized by the sequencing of the human genome, contemporary research now permits efficient investigation of the proteome, transcriptome, microbiome, etc. of any organism for which there is a high-quality genome sequence and annotation (identification of the structure and functional importance of individual genes). Using the horse as an example, the equine reference genome has allowed for identification of genetic variants associated with coat-color, dwarfism, cytokine expression and multiple other traits. Furthermore, the equine reference genome has made possible the Functional Annotation of Animal Genome (FAANG) project, a collaborative initiative promising to correlate and make available a tissue-specific transcriptome (identification of all the genes expressed in a specific cell type) and regulome (all of the regulatory components of a specific cell type) of the horse.

Like "omics" investigations in humans and horses, computational approaches represent a powerful toolset to improve our understanding of S. neurona and EPM. The current reference genome for S. neurona was produced more than a decade ago. Using state-of-the-art, next-generation sequencing technologies and improved computational methodologies, we have generated an updated reference genome for S. neurona that provides an improved assembly and more accurate genome annotation. Importantly, previous gene annotation for the S. neurona genome relied heavily on evidence from the "close" relatives Toxoplasma gondii and Neospora caninum. In this new genome, the structure of each gene has been painstakingly curated using evidence derived specifically from S. neurona, which should ensure the peculiarities caused by 250 million years of Sarcocystis evolution remain evident.

This collaboration between the University of Kentucky's Gluck Equine Research Center, Purdue University and the University of Georgia will give us and other researchers a better understanding of the genetic "language" of S. neurona, which should lead to improved diagnostic methodologies, preventative measures and treatments for EPM.

Learn more about the authors by clicking their names:

Jamie Kaj Norris, PhD Student
Dr. Dan K. Howe, Supervisor
Gluck Equine Research Center, University of Kentucky.

Fourth Quarter 2023

International report on equine infectious diseases.

This report collates information provided by diagnostic laboratories in Lexington, Kentucky,

University of Kentucky Veterinary Diagnostic Laboratory and Equine Diagnostic Solutions, Inc. (EDS). We are also grateful to IDEXX laboratories, Germany, for sharing their quarterly PCR respiratory panel results with this community.

We have further included information from the International Thoroughbred Breeders' Federation, the International Collating Centre (ICC) in Newmarket/Cambridge, United Kingdom, and information from the American Association of Equine Practitioners' Equine Disease Communication Center (EDCC). This report summarizes heightened activity of several relevant contagious or environment-linked diseases among equids. We encourage everyone to report laboratory-confirmed (toxico) infectious disease of Equidae to the ICC or EDCC.

For North America and Europe, there is steady reporting of Strep. equi spp. equi (Strangles) cases. Several North American states and provinces report single cases of EIA (exception: Texas with eight), while there is a single 'Old World' report from Bulgaria.

While the third quarter experienced a true surge of West Nile virus (WNV) infections in California and the U.S. Rocky Mountain states, the situation has cooled a little. New cases have been reported scattered across the U.S. and from the eastern Canada provinces. The same holds for Eastern equine encephalitis virus (EEEV) infections, which decreased during fall/winter due to decreased mosquito activity. The Mediterranean Basin also reports WNV cases (28 from southern Europe and two from northern Africa). Two more endemic regions for WNV have emerged: Berlin region, Germany, and Vienna, Austria.

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EDITORS
Lynne Cassone
Lutz Goehring
Allen Page

STAFF
Holly Wiemers
Hendri Smeenk

Combined, they report close to 10 cases of WNV infections in horses. Interesting is the slow spread of WNV cases into neighboring regions. Noteworthy is the report of two Western equine encephalitis (WEE) virus cases from a bordering region between Uruguay and Argentina.

Incidental Equine Influenza cases/outbreaks have been reported from Ontario, Tennessee and the Pacific Northwest; a few more reports reached us from Northern Europe.

Vesicular Stomatitis virus (VSV (*Rhabdoviridae*)) infections in horses in the U.S. is still very present in California.

As Northern Hemisphere-bred broodmares enter their third trimesters of pregnancies, the season for EHV-1 (EHV-4) abortions has begun, and we received reports of incidental abortions. Cases of EHV-1 neurologic disease (EHM) in North America and Europe have also been reported.

Several countries in continental Europe report outbreaks of Atypical Myopathy (AM), the result of Sycamore seeds and seedling ingestion, which can trigger a severe and life-threatening form of type-1 (oxidative) muscle myopathy. Outbreaks of AM follow a seasonal and severe weather pattern during winter and spring.

Kentucky laboratories and the laboratory in Germany report high incidence of *Strep. equi spp equi* and EHV-1 positive samples. EDS (Kentucky) reports a small number of Equine Influenza positive samples with sample origin from the Eastern United States.

Learn more about the authors by clicking their names.

Lutz Goehring
Edward O. Olajide

Gluck Equine Research Center – Department of Veterinary Science - University of Kentucky

Antimicrobial usage

The discovery, development, clinical use and introduction of antimicrobials into human and veterinary medicine as well as food animal and plant production transformed the management of infectious diseases in the 20th century. Critically, antimicrobials significantly improved the quality of human and animal life. Apart from the effective treatment of bacterial and fungal infections, the effect of antimicrobials is also reflected in increasing meat production yields that has helped to feed an ever-growing human population. Now, the availability and uses of antimicrobials are taken for granted by all who were born after the Second World War.

Sadly, the injudicious, widespread overuse in human as well as veterinary medicine has rapidly led to a precipitous decline in their effectiveness because of rapidly spreading antimicrobial resistance among pathogens. This real and present threat is making antimicrobials ineffective in controlling infectious diseases in human and animal patients. The United Nations Environment Health Programme document, "Environmental Dimensions of Antimicrobial Resistance," quotes, "Antimicrobial resistance (AMR) is already a leading threat to global health and risks adversely affecting the environmental sustainability of the planet."

Equine infectious disease management is now faced with adopting strategies to stop expectations that antibiotics and antifungal medicines should routinely be used indiscriminately for the prophylaxis of infections that may or may not occur as a consequence of viral infections, surgical procedures or stallion and broodmare management. The horse owning public and veterinarians can no longer expect or dispense antimicrobials without evidence that an infection is caused by organisms that are confirmed to be sensitive to a specific antimicrobial treatment. Clinical investigation and diagnostic procedures must be aided by the appropriate laboratory investigations and antimicrobial sensitivity testing. In practice, the dilemma is that this approach is slower than a rapidly progressing infection. The judicious use of a broad-spectrum antibiotic until microbial culture and sensitivity results are available may then be considered as a first treatment step.

Maxwell H. Gluck
Equine Research Center
Lexington, Kentucky USA, 40546-0099
Telephone (859) 257-4757
Fax (859) 257-8542
gluck.ca.uky.edu

The time limiting factors for the clinician are, first, rapid access to clinical pathology laboratory facilities, and, second, requesting the appropriate tests based on a horse's or herd's clinical presentation and symptoms; laboratory professionals often can help guide these requests.

Going forward, animal management and husbandry must be based on best practice biosecurity, stable and personal hygiene, animal movement, vaccination strategies and understanding immune system responses. Rapid recognition of developing infections is an essential part of good horsemanship. This means a return to more intense monitoring of individual horses and herd behaviour patterns. As in the pre-antimicrobial era, being more in tune to changes in appetite, attitude and daily monitoring of rectal temperatures to help identify early signs of illness is necessary. Furthermore, when dealing with both accidental and post-surgical wounds, appropriate topical wound management strategies limit the need for antibiotics. Most cuts and abrasions don't need "antibiotic cover."

Within the equine industry, broodmare and young stock practices have been a major culprit for the indiscriminate and liberal use of large amounts of antibiotics. Critically, strategies to limit antimicrobial use to confirmed cases of post-breeding bacterial endometritis in susceptible mares rather than for general prophylaxis has resulted in a welcome reduction in usage without a discernible impact on per mare cycle conception rates. Additionally, treating healthy newborn foals with a course of prophylactic antibiotics is not necessary and should be reserved for use following the appropriate diagnostic procedures for the treatment of the sick foal. Likewise, the risk of developing respiratory disease ("shipping fever") during and following transportation is not decreased by a course of "preventative" antibiotics. Rather, best practice is to ensure that the horse is healthy prior to travel and closely monitored for a minimum of three days at its destination. The larger risk is that horses treated inappropriately prior to travel will develop resistant strains of pathogens, narrowing the choice of antibiotics for effective treatment when they are truly necessary.

National and international veterinary associations are implementing guidelines for the responsible stewardship of antimicrobials in response to the United Nations' One Health recommendations. These include the British Equine Veterinary Association's "Protect Me Toolkit." It encourages veterinarians and their practices to implement antimicrobial stewardship strategies.

The response to the programme has reduced the use of antibiotics significantly without detriment to the horses in the care of a large general and referral equine practice in Newmarket during the last 10 years. While this progress in antibiotic stewardship by UK equine practices has been substantiated by a recent study highlighting both improvement as well as deficiencies in its implementation, there is still much room for improvement. Taking responsibility for the implementation of antibiotic stewardship policies requires continuous critical monitoring of drug usage, behaviours and trends as well as ongoing education of veterinary professionals and their clients.

In order to protect our ability to manage infectious disease in animals and humans now and in the future, strict antimicrobial stewardship is essential. This is our collective responsibility for all involved in this very diverse global equine industry.

Fred Barrelet, DipVMS (Bern), Dr.med.vet. (Bern), MRCVS
Rossdales Ltd, Newmarket, UK
fred.barrelet@rossdales.com

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Is vaccination against Equine Herpesvirus Type 1 (EHV-1) a rational choice?

"Those who cannot remember the past are condemned to repeat it."
George Santayana, The Life of Reason, 1905"

In the early 1930s, researchers in the Department of Animal Pathology at the Kentucky Agricultural Experiment Station, namely Dimock, Edwards, Doll and others, together with colleagues at Vanderbilt University, Goodpasture and Randall, performed groundbreaking research on an equine abortion epidemic plaguing the industry. They showed in a series of studies that the causative agent was "filterable," i.e., a virus, which they propagated in various experimental models, including infected tissue cultures taken from aborted fetuses, grafts on chorioallantoic membranes of fertilized eggs and Syrian hamsters. The specific cause, a herpesvirus, however, was not confirmed until the 1960s.

Equine abortion virus, as EHV-1 was originally called, caused so much damage that vaccination of horses was attempted before the final identification of the virus. The early trials were done with inactivated preparations after virus amplification in hamsters but were controversial because severe local and systemic reactions ensued. What is more, they were not sufficiently effective against abortion storms within the high-density horse populations of Kentucky. Therefore, live virus preparations were introduced soon thereafter in controlled infection programs in mares. Finally, in the early- to mid-1960s, cell culture adaptation and weakening of EHV-1 strains resulted in the development of modified-live virus (MLV) vaccines, some of which are still in use. It was only in the late 1970s that the planned infection programs were abandoned, thanks to the demonstrable safety and efficacy, albeit limited, of developed vaccines.

In the early 1960's, two main types of EHV-1 vaccines were developed: inactivated (killed) vaccines and MLVs. Bryans and Doll first described the use of inactivated Ky-D, grown in hamsters, for immunization of young horses. At the same time, in Europe, Mayr and colleagues developed an MLV by repeated passage of virus in cultured cells. Interestingly, nothing much has changed principally in the production of these vaccines to this day.

While MLVs are thought to generally provide stronger protection, they may not be suitable for pregnant mares or immunocompromised horses; inactivated vaccines are thought to offer a safer alternative for these sensitive groups.

The vaccination schedules for EHV-1 varies depending on the horse's age and the type of vaccine used. Foals typically receive their initial vaccination at 5 to 6 months old, followed by booster vaccinations every six months until they reach 2 years old. Adult horses require booster vaccinations at least every six months to maintain some level of immunity. In pregnant mares, the only vaccine licensed "to aid in the protection against EHV-1-induced abortion" is to be used three times during pregnancy. In the absence of a radically different approach to vaccine formulations, vaccination habits and schedules are unlikely to undergo a major change.

As old as the vaccines themselves is the controversy regarding the efficacy of vaccination against this virus and its close relative, EHV-4, which during their long co-evolution with their natural host have excelled at the art of immune evasion. In other words, EHV-1, like any other herpesvirus, does everything it can to circumvent and trick the immune response. This makes the development of new and more efficacious vaccines inherently difficult. While countless researchers have tried and failed, we must acknowledge that we still (have to) rely on products that are old but have yet to come of age.

It is, however, accepted by many that vaccination against EHV-1 remains a critical component of responsible horse care and occurrence reduction of the most devastating consequences of the infection, equine herpesvirus myeloencephelopathy (EHM) and abortion.

We would like to think that, by incorporating EHV-1 vaccines into a comprehensive preventive health care plan, horse owners and veterinarians can ensure the health and well-being of our equine companions. Although two recently published systematic reviews and meta-analyses of vaccines and vaccination regimes have revealed that confidence in the value of vaccination is low, it remains the consensus that vaccination is an important tool for reducing EHV-1 spread and the severity of clinical signs.

One may argue that these analyses are worrying – and they indeed are. On the other hand, one can refer to data that have shown a clear decrease in EHV-1-induced abortions in areas where vaccination is widely employed, for example in broodmare farms in Kentucky. While the reduction in cases is hardly the result of vaccination alone, it remains part of a comprehensive approach to maintain the (reproductive) health of operations.

Considering these systematic analyses and findings, one has to recommend continuation of vaccination programs, although it would always be better to have clear and convincing evidence of vaccine and vaccination efficacy. It seems likely we will have to continue to live with the uncertainty as we are, unfortunately, still far away from the astounding efficacies of mRNA vaccine technology, which has been used to control COVID-19. We must be aware of the past limitations of these vaccines and recognize that vaccine development will continue to have an element of trial and error and remain somewhat unpredictable. However, it is clearly worthwhile to continue the quest of finding a more reliable vaccine for EHV-1 and to explore new promising technologies. We must ensure that studies to test vaccines and vaccination regimes are informative and properly done. If we are nimble, the hope will stay alive that, one day, we will have irrefutable evidence that one vaccine or another will do what vaccines are supposed to do: prevent clinical disease, and possibly even infection. It must reman our goal to continue towards a solution that puts owners and individuals at ease and instills confidence that horses in our care are protected against EHV-1 infection and its consequences.

Dr. Klaus Osterrieder
Professor of Virology and Chair
Institut für Virologie, Berlin
no.34@fu-berlin.de

Equine Rotavirus – a 2023 perspective

Since the discovery of equine rotavirus B (ERVB) by the University of Kentucky Martin-Gatton College of Food, Agriculture and Environment's Department of Veterinary Science in 2021, research has been ongoing to develop tools to protect foals against infection. The equine industry has answered the call for financial help, and, along with internal funds from the university, a substantial amount was raised to push forward on vaccine development and antibody synthesis strategies.

Rotavirus is highly contagious with extrapolated data suggesting that only 100's to 1,000's of infectious virus particles are needed to infect susceptible foals. This makes biosecurity a challenge. Measures that farms have employed to break the cycle in an outbreak or prevent infection include foaling outside with personnel wearing personal protective equipment, minimal handling of the foal for the first week of life other than the customary 'foal exam' at 12-24 hours of age and rigorous observance of staff/traffic/horse movement in the foaling barn. Links to additional resources are included below. Control of infection during an outbreak is a considerable challenge, emphasizing the need for a protective vaccine.

One significant hurdle in the development of ERVB-directed treatment and prevention tools is the inability to grow the virus in a laboratory. The currently available equine rotavirus A (ERVA) G3[P12] vaccine was developed from a cell-adapted strain of ERVA. This vaccine has made neonatal ERVA extremely rare when foals are born to appropriately vaccinated mares.

The reason why ERVB replicates robustly in foals but has not been able to be grown in the laboratory remains elusive. This is currently under extensive investigation at the Li-Wang Lab at UK's Gluck Equine Research Center. The importance of cell-specific surface molecular patterns in pathogen and cancer biology research has been increasingly demonstrated. Recent work on specific cell surface markers recognized by different rotaviruses has demonstrated that ERVA and ERVB recognize different cell surface markers with varying affinity. While ERVB does not recognize or interact with the same cell surface glycoproteins as ERVA, promising work has identified molecular elements of cell surface markers for which ERVB has a higher affinity.

These affinities for specific cell surface markers may be used as a screening tool to identify additional cell line candidates in which ERVB could be propagated in the laboratory going forward.

While ERVB cannot yet be grown in the laboratory, vaccine technology has significantly improved since the first equine rotavirus vaccine was developed in the early 1990s. As a result, the need to grow ERVB in the laboratory to generate a vaccine is no longer the insurmountable barrier it once was and several alternative vaccine platforms are currently being explored. Indeed, COVID-19 catapulted vaccine development forward by decades and veterinary medicine is now benefiting from that technology. Vaccine platforms, such as the insect virus baculovirus, can be used as a means to deliver specific components to elicit an immune response, while also being safe for the animals that receive it. Other vaccine technologies, such as mRNA, virus-like particles and expressed protein antigens also show huge promise for the veterinary field. In addition to new antigen formats, the addition of lipid-based nanoparticle adjuvants has improved the effectiveness of modern vaccines considerably compared to older products.

These adjuvants are changing the landscape of vaccine development, which may ultimately result in lower antigenic loads (lower quantities of viral components) per vaccine and longer intervals between vaccinations.

Currently, the University of Kentucky is testing the immunogenicity and safety of four ERVB vaccine candidates; results of these studies will be made available as soon as possible. Given that ERVB was only recognized three years ago, the progress that has been achieved in this relatively short period of time is remarkable and emphasizes the importance of cooperative efforts between the Gluck Equine Research Center and the equine industry in addressing industry problems.

Learn more about the authors by clicking their names.

Dr. Emma Adam
Dr. Feng Li
Dr. Dan Wang

Gluck Equine Research Center
University of Kentucky

These two videos are additional information to this article and by clicking on the images you will be able to watch them on YouTube.