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Symposium



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EQUINE SCIENCE 2010

“Research for the 21st Century Horse Industry: From Genomics to the Winning Post”

The inaugural Australian Equine Science Symposium arose from discussions that were held at the Magic Millions Thoroughbred sales in January 2006. Following initial support from RIRDC, a committee was formed and Equine Science 2006 planned. Following the success of the initial Symposium it was decided by those at the meeting to hold a biennial conference for equine scientists which:

- Promotes excellence in equine science
- Focuses on science, technology and innovation relevant to the Australian horse industry
- Provides a regular forum for exchange of research findings, ideas and information between Australian & New Zealand equine scientists and with their international colleagues
- Assists young equine scientists with their careers
- Encourages participation by members of the horse industry

At the second Symposium a decision was made by the participants to form an Australasian Equine Science Society and the name of the Symposium changed accordingly.

Proceedings

Papers presented at this Symposium have been peer reviewed. However, the comments and views expressed in the papers are entirely the responsibility of the author or authors concerned.

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EQUINE AMNIONITIS AND FOETAL LOSS: PLACENTITIS FOLLOWING EXPOSURE OF MARES TO PROCESSIONARY CATERPILLARS

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Equine Amnionitis and Foetal Loss (EAFL) was first described in 2004 following an outbreak of abortions in mares that had an unusual and consistent pattern of clinical and pathological signs. Examination of reports for all equine abortion cases that involved submission of diagnostic material to the Scone Veterinary Diagnostic Laboratory between March and October 2004 indicated that EAFL was the most common identified cause, responsible for 28 (37%) of 76 cases (Todhunter *et al.*, 2009). The case definition for EAFL (Todhunter *et al.*, 2009) has similarities to descriptions of abortions associated with Mare Reproductive Loss Syndrome (MRLS) reported in Kentucky, USA in 2001 and 2002 (Perkins *et al.*, 2007). MRLS has been shown experimentally to be associated with exposure of pregnant mares to Eastern Tent caterpillars (*Malacosoma americanum*; Webb *et al.*, 2004). A similar hairy caterpillar, *Ochrogaster lunifer* (Processionary caterpillar; PC), was present in large numbers on several farms where EAFL abortions were diagnosed.

Experimental induction of EAFL: A series of experiments were conducted to determine the possible role of PC as an abortifacient in mares. The initial experiments were designed to simulate the situation in the field where most EAFL cases occur in mid to late gestation. The results showed that exposure of mares to preparations of either whole PC or shed PC exoskeleton can induce pregnancy loss (Cawdell-Smith *et al.*, 2007). Gross pathology and bacteriology results were similar to those seen in field cases of EAFL (Todhunter *et al.*, 2008). Of additional interest was the finding that some showed gross pathology that was very similar to that of Nocardioform placentitis (Cawdell-Smith *et al.*, 2010).

It is well recognised that PC can cause an intense allergic reaction in humans. In the experimental studies, some but not all mares displayed a transient urticarial reaction following exposure to PC. In the studies using exoskeleton preparations, urticaria was not a predominant sign, although it did occur in some mares (Cawdell-Smith and Bryden, 2009). The mares exposed to exoskeleton preparations showed no detectable clinical abnormalities. A number of the abortions in these mares were acute but other outcomes were abortion with placental and foetal finding consistent with chronic infection as well as delivery of live but affected foals. In these cases, findings indicate that the initial insult occurred some time prior to abortion or birth.

The other distinctive feature of the field syndrome (Todhunter *et al.*, 2009), and also observed in the experiments (Cawdell-Smith and Bryden, 2009), was the type of bacteria isolated from aborted fetuses. The range of organisms found was similar in both the experiments and in field cases. The organisms were common environmental or intestinal bacteria that are not normally associated with illness in horses. Although there were changes in haematology in some mares that were suggestive of a mild infection, those findings were not consistent. The isolation of bacteria and the pathology of the foetus and placenta were consistent with infection in the foeto-placental unit. This finding supported the conclusion that abortion occurs following passage of normal gut and environmental bacteria through the gut wall into the mare's blood stream, and then via haematogenous spread to the foetus.

The occurrence of mid-term abortions coincides with the migratory behaviour of PC and it was of interest to determine if these caterpillars could also cause either early embryonic loss (<35 days gestation), or abortion in mares from 45 – 60 days of gestation (Cawdell-Smith *et al.*, 2008). While one treated mare did abort in the early embryonic loss trial, the result was

inconclusive. However, it was shown that mares exposed to PC exoskeleton during the early foetal stage (45 – 60 days gestation) may abort.

Caterpillar exposure and possible mechanism of action: The studies described above demonstrate that exposure of pregnant mares to PC can induce foetal loss. However, it is well known that horses are able to sift through feed to avoid eating material that they do not wish to ingest. It is unlikely that horses would consume whole caterpillars. However, it is likely that as shed exoskeleton is extremely light (one exoskeleton weighs approximately 0.01g) and is easily blown around the pasture, it would be difficult for it to be separated from pasture by grazing mares.

It would appear from our results that structural elements of the caterpillars' body, more specifically those associated with the exoskeleton, or a compound in the caterpillar, or a combination of the two, allows the transfer of bacteria across the mare's gastrointestinal wall and the placenta. The resulting infection in the foetus leads to foetal death and then abortion. The PC species have hairs or setae that are barbed and it was thought possible that ingested setae penetrate the gut wall. This has recently been demonstrated in mares intubated with PC (Todhunter *et al.*, 2010). The question then arises as to how easily bacteria cross the gut wall and the placenta. These caterpillars may also contain a toxin(s), which is consistent with their uticular action, that may affect the gut and placenta and facilitate transfer of bacteria.

In conclusion, examinations are currently being conducted of histopathological changes in maternal, placental and foetal tissue samples from mares that have been treated with PC in an attempt to better understand the role of setae and/or toxins in the pathogenesis of EAFL. A laboratory animal model of EAFL is also being explored as an efficient alternative to using pregnant mares to allow detailed, ongoing studies of this condition. Moreover, there is justification for further studies that seek a greater understanding of the life cycle and population dynamics of processionary caterpillars, and of the caterpillar-associated factor(s) that induce abortion in mares.

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NMR-BASED METABONOMIC ANALYSES: A SEARCH FOR METABOLIC MARKERS FOR GENETIC PREDISPOSITION TO OCD

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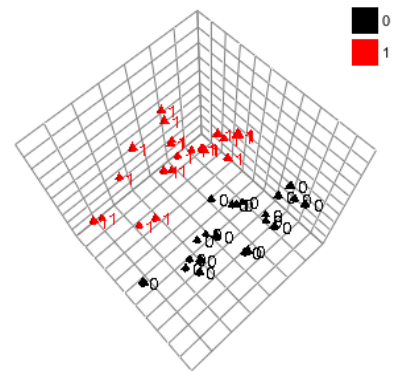
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Metabonomic investigations use complex multivariate statistical analyses of Nuclear Magnetic Resonance (NMR) spectra from biological samples such as serum, saliva and urine to detect novel metabolic markers associated with diseases. The detection of significant differences in a distinct array of metabolites from diseased and normal individuals can lead to the development of new diagnostic and therapeutic modalities based on deeper understanding of the metabolic processes involved. Osteochondrosis dessicans (OCD) is a developmental orthopedic disease that has been well documented to have a genetic basis in Standardbreds and other breeds but the actual metabolic defects causing the lesions have not been well defined. The occurrence of OCD lesions was correlated with hyperinsulinemic responses to glucose challenges in weanling Standardbreds but not proven to be a true causal factor. Serum and plasma were collected on a single day in August, 2007 (n=48) and two days in 2008 (n=75) from yearling Standardbreds housed at a single facility. Metabonomic analyses of the NMR spectra of the serum samples revealed clustering (Figure 1, $P<0.05$) due to repeatable differences in the metabolic profiles of horses that had had OCD lesions surgically corrected (OCD, n=54) 2 to 9 months before sample collection versus those with the same sire, similarly bred dams and raised on the same farm with the same nutritional and environmental management that did not have lesions (Control, n=69, 22 of which were radiographically verified). There were no differences ($P>0.05$) in total plasma glucose or insulin between the two groups and no clustering based on date of surgery was observed.

Figure 1: Loadings plot of Orthogonal Partial Least Square-Discriminate Analyses of NMR spectra of serum from Standardbred Yearlings in which presence (1, n=22) or absence (0, n=22) of OCD lesions was verified radiographically.

Metabolites which differed ($P<0.05$) between OCD and Control horses included alpha and beta glucose, phenylalanine, choline, threonine, valine and various lipids.

NMR based metabonomic analyses were able to reveal significant differences in a variety of metabolites in yearling horses that developed OCD lesions versus half or full siblings that did not develop lesions, despite having had the same nutritional and environmental management since birth. Based on these results, it appears that there are distinct metabolic differences in young Standardbred horses that develop OCD versus those that do not. This will aid in genomic identification of the defective metabolic pathways in genetically predisposed foals and perhaps used to detect foals at risk before lesions appear. Once this is accomplished therapeutic modalities may be explored to prevent lesions in foals at risk. Further studies are in progress to determine if the metabolic differences observed are present in foals before lesions appear.



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TO WEE, OR NOT TO WEE; GENETIC IDENTIFICATION OF THE DONORS OF DRUG-POSITIVE URINE SAMPLES

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Confirmation of identity and parentage by DNA profiling is routinely carried out in the Australian horse industry and around the world by DNA profiling using sets of DNA microsatellite markers. This confirmation of identity is mostly used for horse breeding, registration and import and export purposes. The Australian Equine Genetics Research Centre (AEGRC) mainly extracts DNA for profiling from horse hair, but sometimes from other samples such as blood or semen. Methodologies to extract DNA from these samples are fairly advanced, with DNA profiling achieved, usually one to two weeks.

DNA profiling from urine has been achieved in human forensics, and in some cases from horses, but extraction of DNA from urine samples in a consistent and reliable manner has been elusive in both humans and animals. Extraction of DNA from urine has proved difficult due to the small amount of cellular material present and the degraded state of the DNA that is present. The amount of DNA in urine also shows large variability between samples and urine contains many substances which inhibit amplification of extracted DNA. The research in this project examined methodologies to consistently obtain reliable DNA profiles from horse urine and to investigate how a process for genetic identification of urine samples could be integrated with horse racing industry drug testing procedures across Australia.

Methods: Different methods for DNA extraction were tested on horse urine samples. These included published methods, commercially available kits, unpublished methods communicated from other international equine DNA profiling laboratories, and modifications and combinations of all of these. Different volumes of urine were tested along with the effects of storage temperature and time on DNA extraction and amplification success. Extracted DNA for a set of twelve equine DNA microsatellite markers and analysis of amplification results was carried out according to the AEGRC standard operating procedures. These markers include nine DNA microsatellites recommended by the International Society of Animal Genetics to the International Stud Book Committee, and three extra markers are routinely used by AEGRC. Determination of the DNA profile from any sample of biological material involves amplification of a multiplex fluorescent PCR system, capillary electrophoresis and analysis by Genemapper software (Applied Biosystems). The AEGRC's DNA profiling system for horses has been certified under an ISO9001-accredited quality management system since 2006.

Results: The success of DNA profiling horse urine samples was influenced by the time and method of sample storage, and by the method of DNA extraction. Initially, utilising centrifugation, saline washing and a commercially available DNA extraction kit (Gentra Puregene), the genotypes of eight out of twelve microsatellite markers were able to be determined from a horse urine sample, called a "Partial DNA Profile". The partial DNA Profile determined from the urine sample matched the DNA profile obtained from a hair sample of the same horse. This study also found some success with DNA extraction from urine using hydrochloric acid (HCl) to remove insoluble calcium carbonate and calcium oxalate. These substances sediment with cellular material and inhibit extraction of DNA. Using this method a partial DNA profile of nine out of the twelve DNA microsatellite markers was obtained from a horse urine sample. Again, these markers matched those from a hair sample from the same horse. However, the most successful method used was a recently developed commercially available kit, QIAamp Micro Kit (Qiagen). Using this kit complete, or full DNA profiles with twelve complete markers were accurately determined.

Variability between urine samples seems to be an ongoing issue. In this initial study we tested seven urine samples. Four of these samples have produced full DNA profiles with twelve complete markers, and the remaining three samples produced partial DNA profiles. This variation is likely to be contributed to by excess handling and repeated freeze-thawing, or it could be due to different shedding rates of epithelial cells between different individuals. In humans, female urine samples are seen to contain more cellular material than male samples, but this has not yet been established for horses.

Storage time and temperature were found to have a significant effect on the DNA profiling of horse urine samples. Successful DNA profiling has only been achieved from samples stored at 4°C for not more than two days and then extracted and profiled, or from urine samples stored at -20°C or -80°C after storage at 4°C for not more than two days. No profiles were obtained from samples that were stored at 4°C for a week or longer. Moreover, the volume of urine used was also found to have a significant effect on the ability to obtain a DNA profile. Although very small volumes of urine have been reported to give a DNA profile in human studies, the results in this study suggest that at least 1 ml of urine is needed to obtain DNA profiles from horse urine samples.

Implications: The results of this study provide promise that DNA profiling from urine is close to becoming a procedure that can be offered to confirm the identity of drug-positive samples. However, due to the nature of the urine samples and variability in storage conditions there are issues that will need to be addressed with relevant stakeholders. Australian horse racing regulatory authorities and drug-testing laboratories will be major stakeholders for this test. Volumes of urine required and storage conditions of urine will need to be discussed with the drug-testing laboratories, as these factors will affect their collection procedures and sample storage protocols.

Nevertheless, the progress made is significant and the ability to determine accurate and complete DNA profiles from horse urine samples provides confirmation of the identity of the donor of a urine sample with a confidence level of greater than 1 in 100,000,000,000 (1 in 100 billion). In human forensics, with degraded or difficult samples like urine, forensic samples in which the full complement of markers have not been obtained are still of use in court cases, as even with a smaller number of markers (a partial DNA profile) the power of confirmation of identity with DNA profiling can still be very high.

DNA profiling to confirm donor identity of horse urine samples has significant benefits to offer trainers and owners, as well as racing regulators and drug testing laboratories. Importantly, genetic confirmation of the identity of the donor of a drug-positive urine sample has the power to exonerate the innocent, and owners and trainers need to be aware of the availability and power of this genetic technology.

Extending the use of DNA profiling, from confirmation of bloodlines and identification of imported horses, to include confirmation of donor identity of urine samples collected for drug testing would be another milestone in establishing and maintaining public confidence in, and ensuring the integrity of the Australian horse racing and breeding industries.

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UPDATE ON EQUINE OBESITY AND ISSUES WITH MANAGEMENT

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We all are aware that the number of people, especially children, with obesity (defined as the excessive accumulation of adipose tissue (fat) in the body) has increased dramatically recently, to almost epidemic proportions in some countries. Obesity in man increases the likelihood of developing Type 2 diabetes, hypertension, coronary heart disease and premature death. In the horse obesity, insulin resistance (IR) and hyperinsulinemia have recently been associated with an increased risk for laminitis. The term ‘equine metabolic syndrome (EMS)’ has been used to describe the clustering of obesity (generalized or regional), IR, and prior or current laminitis. Obesity also may be associated with reduced performance, heat intolerance, increased bone/joint injuries, altered growth patterns as well as an increased risk of DOD. In a study of 319 pleasure-riding horses (Wyse et al 2008) in Scotland, 10% were considered obese and a further 35% fat. Currently there is no universally accepted definition of obesity in horses/ponies and no general validated body condition scoring system. As with humans there may be an association between regional adiposity and disease risk e.g. neck crest adiposity (Carter et al 2009a,b).

Most cases of obesity are associated with an imbalance between energy intake and expenditure. Intriguingly nutrition during gestation may also influence the predisposition to obesity in later life in the horse as for other species. However, there also may be breed and seasonal effects e.g. native UK pony breeds exhibit appetite seasonality and under ‘feral’ conditions tend to gain weight during the summer when food is abundant before losing it during the winter. Such cyclical changes may not occur when food intake and quality is maintained during the winter resulting in further weight gain. Pronounced differences were reported recently according to season and initial BCS on weight gain when Welsh Mountain ponies were fed *ad libitum* a fibre-based ration (Dugdale et al 2010a).

Key practical management strategies of obese/overweight animals include: 1) promotion of weight loss and improved insulin sensitivity via **dietary restriction** and, where possible, an **increase in physical activity**; 2) avoidance of feeds that may exacerbate insulin resistance and hyperinsulinemia (feeds rich in non-structural carbohydrates such as grains, high starch containing feeds and ‘lush’ or stressed pasture forages).

Any weight loss programme needs to be **targeted to the individual animal** but to have a chance to work requires

- Most importantly, recognising that the animal is overweight, that it needs to lose weight and that this will take time and effort.
- Understanding exactly what is currently being fed (Scoops and haynets weighed etc. Analysing the forage may be required– or use low energy forage replacers). Note large amounts of grass can be ingested in a short turn out time (Ince et al 2005).
- Being realistic about the amount and type of work the horse actually does and can do. This may require veterinary consultation.
- Establishing a programme to promote weight loss – this may just require restricting grazing, changing the type of forage fed or using a low energy forage replacer and/or reducing the amount or change the type of complementary feed provided – whilst maintaining vitamin and mineral intake to support health. For some individuals more severe restrictions may be required e.g. as a general guide, for the more obese animals or for those where appropriate changes in the ration as outlined above have not been successful, consider providing low energy hay or forage substitutes initially at ~ 1.5% of current BW daily (on a Dry-matter intake) with subsequent further reductions if required

but recommend not to decrease to less than 1.0% BW (DMI); feeding reduced amounts of fibre may increase the risk for hindgut dysfunction, stereotypical behaviours, gastric ulcers, coprophagy etc. The ration should be divided throughout the day and strategies to prolong feed intake time should be considered, (e.g. haynets with multiple small holes). It is very important to maintain appropriate vitamin and mineral intake.

- Do not attempt to make rapid changes to the horse's weight – the maximum amount we would recommend you aim to achieve is 1% body weight per week after the first week, when any weight loss may be due to reduced gut fill, i.e. aim for around 20 kg for a 500 kg horse over 4- 6 weeks. A more realistic (but often still difficult to achieve in the more resistant cases) target is a weekly weight loss of 0.5% (again after the first week).
- Set realistic targets and monitor (under identical conditions) the horse's weight and condition on a regular basis i.e. every 2 – 4 weeks (NOTE that appropriate levels of weight loss may not always be accompanied by significant change in body condition score in the first few months (Dugdale et al, 2010b)).
- Make all dietary changes gradually and avoid prolonged periods of feed withholding. Abrupt starvation especially in obese ponies, donkeys, and miniature horses (especially pregnant animals) carries the risk for hyperlipemia.
- Developing, and continually updating, an appropriate weight maintenance program once the target weight and body condition have been achieved to avoid a return to weight gain.

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AN OVERVIEW OF HENDRA VIRUS

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Introduction

Hendra virus (HeV) and Nipah Virus (NiV) form the two known members of the genus *Henipavirus*, within the Paramyxovirus family. Henipaviruses are the only zoonotic paramyxoviruses, are highly pathogenic, and can infect a wide range of species (Eaton et al 2006).

Host species

Fruit bats or flying foxes of the genus *Pteropus* are considered natural reservoirs of HeV. There are four species of fruit bat in Australia that collectively range across coastal areas of the entire northern and eastern parts of the country (spectacled fruit bat or *Pteropus conspicillatus*; black fruit bat or *P. alecto*; little red fruit bat or *P. scapulatus*; and grey-headed fruit bat or *P. poliocephalus*). Blood samples from all four species have contained antibody to HeV (seropositive) and all species are considered capable of carrying and shedding HeV.

Bats are considered to be carriers of the virus and there is no evidence that HeV causes overt clinical disease in bats.

A range of animal species have been exposed to HeV in experimental studies. Mice, rats, rabbits, chickens and dogs did not develop disease following inoculation with HeV, although rabbits did develop neutralising antibody titres and some dogs and rats developed equivocal titres (Westbury et al 1995). Experimental infection of cats, ferrets and guinea pigs resulted in animals developing disease and cats and ferrets have subsequently been used as animal models for studying HeV infection under experimental conditions (Westbury et al 1995; Williamson et al 2000; Weingartl et al 2009).

Ecology

Bats tend to roost on tree branches in large communal camps and leave these camps at dusk or early evening to forage for fruit and flowers, returning in the early morning (Eaton et al 2005). In large, dense roosts or camps bats excrete urine or faeces throughout the day and a fine mist of urine is reportedly commonly observed under these roosts at times. Transmission from bat-to-bat is thought to occur through exposure to these excretions or through social interaction and shared feeding behaviours.

Ecosystem changes including increasing density of human and domestic animal populations, destruction of natural landscape for urban and agricultural development, climate change, disruption in bat populations and changes in bat population dynamics and increased stressors on bat populations (nutritional, reproductive, disease etc), are all considered to be contributing to susceptibility in bats to infection with HeV (Plowright et al 2008).

Bat populations in general are declining in the face of these broad ecosystem changes and bat colonies are becoming smaller and more dynamic. Even though the overall bat population is declining, the presence of increasing numbers of small, highly mobile bat colonies in peri-urban areas may give the perception that bats are more prevalent.

HeV appears to cause discrete mini-epidemics in individual bat colonies as infected bats arrive and introduce HeV into a susceptible colony (Field et al 2007). Epidemic infection in a colony results in increasing levels of virus secretion and increased risk of spillover of infection to horses. Infected colonies develop immunity and virus circulation in that colony is

either reduced or ceases. Over time immunity in the colony wanes and the colony becomes susceptible to re-introduction of virus with another infected bat.

The large number of geographically dispersed colonies in Australia mean that epidemics may be occurring in disparate areas at any point in time.

It is also interesting to note that there have been no confirmed cases of HeV in horses south of Murwillumbah in northern NSW, and all other confirmed horse cases have occurred in coastal areas of Queensland. However, seropositive bats have been identified in WA, NT, QLD and NSW.

Transmission

Infected foetal fluids and placenta as well as other biological fluids (urine, faeces, saliva) all appear to offer potential transmission pathways from bat-to-bat and from bat-to-horse (or other animals).

The mechanism of transmission from bat-to-horse for HeV is assumed to be associated with ingestion of or mucous membrane exposure in horses to virus contaminated fluid or objects such as fruit, spats, and possibly other material (water, feed) contaminated with urine or saliva or placental fluids from infected bats.

Horse-to-horse transmission is considered likely to have occurred in a number of incidents involving multiple horse cases at one location, including incidents where horses have been confined in stables, small yards or paddocks (Hendra, Redlands, Proserpine and Cawarral). Alternative explanations for exposure of other horses at these locations do exist including separate bat-to-horse transmission and human-assisted transmission.

There is also a possibility of exposure via contamination of the immediate environment (urine or ground water, stable walls, doors) or equipment and the possibility of inadvertent human-assisted transmission through use of equipment on an infected horse and then subsequently using the same equipment on another susceptible horse.

The precise mechanism(s) of transmission from horses to humans are not known. All human cases of HeV have involved people with direct and close contact with horses and particularly close contact with horses known to have been infected with HeV. Airborne droplets or splashing of infectious material are considered likely to have occurred in some cases including practices such as flushing nasal cavity lesions under pressure or movement of fluid through an endoscope placed in the upper respiratory tract.

There is no evidence to support direct bat-to-human transmission for HeV infection moving from bats to humans. Human risk under natural conditions is therefore limited to pathways involving human exposure to infected horses or material from infected horses.

Clinical disease in horses

The incubation period (time from exposure to appearance of first clinical signs of disease) in horses is 5 to 16 days. Fatally infected horses died on average two days after the first signs of infection. About 25% of confirmed infected horses to date have survived the acute infection though all of these animals have subsequently been euthanased because of the potential risk to other animals and people.

The most detailed information on clinical disease in horses comes from experimental studies performed at the Australian Animal Health Laboratory (AAHL) in Geelong under the direction of Dr Deb Middleton (Middleton et al 2009). The first change noted was a rise in body temperature and heart rate that appeared between 5-7 days after horses were exposed to HeV. Within 1 to 2 days of the first rise in temperature and heart rate the horses had started to

show overt clinical signs of illness including restlessness, depression, reduced appetite and onset of other clinical signs including respiratory and neurological signs.

The results of this trial confirmed unequivocally that infected horses began to shed small quantities of virus days prior to the onset of any clinical signs and that viraemia, with associated widespread dissemination of virus through the body, was occurring prior to the initial rise in temperature which itself occurred prior to the onset of any overt clinical signs.

Quantitative counts of viral genetic material in samples using qRT-PCR indicated that the amount of virus being secreted was steadily and rapidly rising from the time of first detection and was likely to peak as horses were maximally ill and around the time of death. These results indicate that HeV infected horses pose a risk of being infectious to other animals and people from some days prior to the onset of clinical signs and that the risk then continues to rise in association with the amount of virus being shed and present in tissues as the horse progresses through clinical disease.

Horses infected with HeV have been shown to present with a diverse array of clinical signs, particularly very early in the progression of disease. It is believed that the virus is operating at the cellular level through the same broad mechanisms described above (endothelial cell damage in small vessels and respiratory epithelial cell damage) and that the particular clinical signs and progression of overt disease may depend on individual animal susceptibility and which organs or tissues are subject to the greatest impact of viral infection. The range of clinical signs has been well described in the *Guidelines for veterinarians handling potential Hendra virus infection in horses* (The Guidelines)¹.

Human cases of HeV

All confirmed cases of HeV in horses have been associated with potential for exposure of people. Queensland Health has been responsible for assessing exposure risk in people that had opportunities for interaction with horses on properties where horses have been confirmed with HeV (McCall 2010).

Reports from investigations of the Redlands and Cawarral events suggested that there were between 12-20 individuals at those locations that had sufficient interaction with infected horses to be at either low to moderate, or high risk of having been exposed to HeV in some way. There were three confirmed human cases (all from the high risk exposure groups). When compared against the estimated total of individuals with opportunity for exposure, the proportion of people with exposure risk that became infected was about 10% (range from 8 to 12%). This information suggests that HeV is not highly infective for people and that an individual may have to be heavily contaminated or exposed to virus or be exposed in a particular way in order to be at high risk of subsequent infection.

In all confirmed cases of human HeV infection to date, descriptions of events suggest that individuals may not have implemented the full combination of PPE and other biosecurity measures generally recommended for minimising HeV exposure risk at the time they were likely to have been exposed to infectious material. In the first three cases of human infection this was before any knowledge of the existence of HeV.

There is also a range of evidence to support the suggestion that PPE and additional biosecurity measures may be effective in minimising or preventing exposure to HeV when people are interacting with horses that may be infected with HeV.

¹ http://www.dpi.qld.gov.au/4790_13371.htm

Virus survival

HeV is susceptible to a wide range of disinfectants and detergents and can be effectively destroyed with relatively simple hygiene procedures (hand washing and disinfection).

There is relatively little specific information on henipavirus survival under various conditions. A recent publication provides limited information on this topic (Fogarty et al 2008). HeV can survive in a broad range of pH solutions (4 to 11 pH), is highly susceptible to dessication and rapidly destroyed under direct sunlight but can survive for days under optimal conditions (moist fruit juice or bat urine that is not in sunlight).

These findings suggest that if HeV were secreted into the environment in urine or other secretions from an infected horse for example, that there may be situations where virus survival could be sufficient to pose risk of exposure and infection to other animals. These would likely involve moist areas, mild climatic conditions and areas unexposed to direct sunlight.

Therapeutic options

There appear to be no effective and safe therapies available currently for treating patients with either a high risk of exposure to prevent infection or those patients that are infected based on some form of positive test to HeV.

A variety of therapies have been administered to HeV infected people to date.

Anti-viral medications such as Ribavirin and Chloroquine have shown some beneficial effect in vitro against HeV and have been used to treat humans at risk of infection but the benefits of such treatments remain unclear (Pallister et al 2009).

Recent and ongoing research has focused on two broad approaches to therapeutics (Bossart et al 2007).

The first is the development of vaccines aimed at stimulating immune response directed against specific viral proteins necessary for virus attachment to cell surface receptors and fusion, respectively (Eaton et al 2005; Mungall et al 2006; Weingartl et al 2006; Bossart et al 2007; McEachern et al 2008).

Vaccination may be applied to animal hosts such as the bat (reservoir host) or to intermediate hosts such as the horse or pig in an attempt to reduce the likelihood and amount of virus shedding by animals and as a consequence reduce human exposure risk, or to humans to prevent human disease by developing effective immunity in vaccinated people so even if exposure were to occur that infection would not result.

An effective vaccine applied to either horses or humans, offers potential to provide a major advance in risk management for HeV in Australia. There are major challenges to be overcome before a product may be available for use in Australia and there are considerable uncertainties over efficacy (how good might a vaccine be at protecting against infection) and timeline (how soon might a vaccine be available).

The second broad approach to therapeutics is the development of treatments that may be applied to people after they have been potentially exposed to infectious material to remove or impede the ability of virus particles to establish infection (post-exposure treatment). Examples include antibodies, fusion inhibitors and soluble receptor molecules (Bossart et al 2007). Most efforts appear to be directed towards antibody production.

There are a number of studies demonstrating protective effects of monoclonal antibodies directed against henipavirus antigens and administered to animals either before or after experimental challenge with NiV or HeV (Zhu et al 2006; Zhu et al 2008; Bossart et al 2009; Guillaume et al 2009). Preliminary results of some studies indicate that there may be a very

limited time window post exposure when these treatments may be expected to protect against development of disease. Further work is currently being directed at delineating this window of effective treatment and further developing this modality as a potential treatment option for people who have been exposed to potentially infectious material.

Risk management

The absence of effective therapy for HeV infection in people and the high case fatality rate reinforces the importance of infection control precautions and prevention of horse-to-human transmission (Playford et al 2010). These issues are further complicated by the variable and non-specific clinical presentation of horses infected with HeV and the consequent difficulties in diagnosing HeV or even recognising that HeV may be a possible cause of disease in a presenting horse. Finally there is the small but real risk of horses that are infected with HeV but not yet displaying any clinical signs of disease (in the late stages of incubation) also presenting exposure risk to people.

Efforts at developing preventive therapies including vaccination, are supported while recognising that the most critical challenge facing the Australian horse communities is the implementation of effective risk management based on PPE, personal hygiene and other biosecurity measures to protect animal health workers and horse owners today given that the next case of HeV may be occurring now.

Routine adoption of infection control precautions are therefore critical to minimising exposure risk when interacting with horses. The range of precautions includes hand washing, use of gloves and other forms of PPE and a high index of clinical suspicion of HeV (Playford et al 2010).

Risk management may be broadly divided into different levels – routine management to be adopted when ever people are interacting with horses, precautions to be implemented when performing procedures that may be associated with exposure to biological fluids or tissues and particularly procedures involving the nose, mouth and respiratory tract, and finally procedures associated with horses that are displaying signs of illness including sudden death.

Guidelines for veterinarians handling potential Hendra Virus infection in horses

The main source of current information about Hendra virus in horses and about managing exposure risk for veterinarians dealing with horses, is the document titled: *Guidelines for veterinarians handling potential Hendra Virus infection in horses*, available for download from the Queensland Department of Primary Industries and Fisheries website.

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PATHOLOGY OF MARES ABORTING DURING EQUINE AMNIONITIS AND FOETAL LOSS

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Equine Amnionitis and Foetal Loss (EAFL) is the term used to describe cases of unusual abortions first observed in Australia in 2004 (Todhunter *et al.*, 2009). Subsequent experiments have shown that exposure of pregnant mares to whole processionary caterpillars (PC; *Ochrogaster lunifer*) or their exoskeleton causes abortion in horses (Cawdell-Smith *et al.*, 2009). The histological aspects of foetuses aborted due to EAFL have been extensively investigated through post-mortems both in the field and experimentally (Todhunter *et al.*, 2009). However, it is not known how ingestion of the caterpillar actually causes the abortion or the effect, if any, on the mare's gastrointestinal tract. This project was undertaken to investigate the pathological changes in the pregnant mare after exposure to whole PC.

Four mares were euthanized and post-mortemed during an experimental trial of gavage with macerated whole PC including one untreated control. Samples were taken for histopathology from specific areas of the gastrointestinal tract, reproductive tract, organs, and lymphatic tissue. These samples were placed in 10% buffered formalin and allowed to fix. Small sections from each sample were selected and processed in a routine fashion to make H&E stained slides. Systematic examination under light microscopy was performed to note the presence of setae within any tissues and the subsequent reaction if present.

Ingestion of the caterpillars created a range of lesions within the treated mares. Setae were found throughout the gastrointestinal tract, concentrated within the caecum and large and small colon. Setal fragments were found in the mesenteric lymph nodes and uterus of one mare, and creating a neutrophilic reaction in the serosa of the uterus in another mare. Within the large intestine, setal fragments were present within the lumen and intima of arteries, veins, and lymphatics of the submucosa as well as sporadic findings within the muscularis and serosal layers of the intestine. Inflammatory reactions around setal fragments ranged from unapparent to neutrophilic (microabscess), eosinophilic, lymphocytic, plasmacytic, and/or granulomatous with multinucleated giant cells. Physical presence within the villi of the large colon of one mare caused an acute right dorsal colitis. Setae were not found in the tissues of the untreated control mare.

These results show that caterpillar setae can cause a wide spectrum of reactions within the equine gastrointestinal tract ranging from unapparent reaction to acute necrotising inflammation to chronic granulomas. The presence of setal fragments within the lymphatic channels in the mesenteric lymph node of one mare supports the theory of setae as foreign body emboli within the lymphatics. Migration of setal fragments is demonstrated by their presence in all layers of the large intestines including the serosa causing a hyperplastic serositis as well as within the uterine serosa and glands. This reinforces the need to limit the exposure of horses to processionary caterpillars with an emphasis on pregnant mares due to the possibility of migrating setal fragments causing gastrointestinal disease or other sporadic diseases including gastroenteritis, peritonitis, abortion, and possibly placentitis.

Todhunter KH, Perkins NR, *et al* (2009). *Australian Veterinary Journal*.87:35-38

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NOCARDIOFORM LIKE FOCAL MUCOID PLACENTITIS AS SEQUELAE TO CATERPILLAR EXPOSURE IN MARES

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Focal mucoid placentitis is a term used to describe the distinctive gross lesions on the placenta of mares caused by nocardioform organisms and historically called nocardioform placentitis¹. The characteristic lesion is a well circumscribed thick, sticky, tan to grey exudate on the surface of the chorioallantois in the region of the union of the horns and body. *Rhodococcus rubropertinctus*², *Crossiella equi*^{3,4}, and *Amycolatopsis spp*⁵ are nocardioform actinomycete bacteria more recently implicated as causative organisms. *Cellulosimicrobium cellulans* (formerly *Oeskovia xanthineolytica*) was first described in Australia in 1982⁶ and later in Kentucky⁷ as causing mucoid placentitis in mares. *C. cellulans* has also been isolated in cases of confirmed Equine Amnionitis and Fetal Loss (EAFL)⁸ which has subsequently been demonstrated to be induced by ingestion of processionary caterpillars (PC)(*Ochragaster lunifer*)⁹. Three cases of focal mucoid placentitis caused by different organisms in association with exposure to caterpillars and satisfying the EAFL case definition criteria¹⁰ are described here.

Case 1 was a mare presented for pre-term labour during the initial investigative outbreak of EAFL. Gross placental lesions were consistent with focal mucoid placentitis and *Stenotrophomonas maltophilia* was cultured from the lung and stomach contents of the euthanased foal. Case 2 was a pre-term birth subsequent to an experimental investigation of maternal PC exposure via gavage in the early embryonic period. Gross lesions were consistent with focal mucoid placentitis and *Stenotrophomonas maltophilia* was isolated from the mucoid material on the chorioallantois. Case 3 was a teaching mare which was bred and placed in a pasture containing Eucalypts trees which were subsequently shown to contain PC nests. The mare had a late-term abortion with a lesion of focal mucoid placentitis on the pregnant horn. *Enterobacter cloacae* was isolated from the placenta and foetus. Recently, caterpillar setae have been found histologically within the uterus of mares experimentally gavaged with PC¹¹. These findings in addition to the commonality of *C. cellulans* isolation, the coexistence of lesions in the fetal membranes pathognomonic to EAFL, and a focal exudative lesion of chronic active mucoid placentitis suggests the route of bacterial entry may be due to caterpillar setae migration. The mare's stage of gestation at the time of caterpillar exposure may cause different pathologic lesions with the same aetiology.

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PLACENTITIS IN THE DAMS OF FOALS ADMITTED TO A NEONATAL INTENSIVE CARE UNIT: CLINICAL SIGNS AT ADMISSION, DIAGNOSIS AND OUTCOME

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Placentitis is a critical determinant of neonatal foal health. Premature delivery, dysmaturity in full term foals and delivery of a small for gestational age foal is associated with clinical placentitis. However there are no recent reports of the clinical presentation, diagnosis and outcome of neonatal foals, delivered from mares with placentitis that were admitted to a neonatal intensive care unit.

Foals (n=86) less than 72 hours old admitted during 2009 to the Scone Equine Hospital Intensive Care Unit (ICU) were analyzed. All placentas were examined and recorded with the foal admission clinical parameters, treatment, and diagnosis and survival data in a spreadsheet. All foals were assessed by veterinarians who are Specialists in Equine Medicine and experienced in the care of neonatal foals. The data was transferred to a statistics program for analysis (JMP 7, SAS Institute Inc, Cary NC, USA). Contingency analysis, Fishers Exact Test, Wilcoxon/Kruskal-Wallis Tests or Logistic Regression was used as appropriate.

Abnormal placentas were recorded for only 17 of the 86 foals (19.8%) admitted to the ICU. Foals admitted to the ICU that were delivered from mares with a normal placenta were more likely to be older at admission to the ICU than foals from mares with placentitis ($p=0.001$, unit OR 1.3). All foals from mares with placentitis were admitted to the ICU before 9 hrs of age. The probability that a foal had a gestational age of <320 days was greater if the mare had placentitis than if the mare had a normal placenta ($p=0.028$, RR 3.82, 95% CI 1.3-11.7). The probability that the delivery was complicated by dystocia was greater if the mare had placentitis compared to mares with normal placentas ($p=0.0015$, RR 2.29; 95% CI 1.5-3.5) There was no association between the presence of placentitis and gender, the length of hospital stay or the assessment of the foal as dysmature.

Foals from mares with placentitis were more likely to have a diagnosis of perinatal asphyxia syndrome ($p<0.001$, RR 1.9, 95% CI 1.5-2.3) than foals from mares with a normal placenta. There was no association of placentitis with positive blood culture, pneumonia, fractured ribs, renal failure, infectious gastrointestinal disease, umbilical infection, uroperitoneum, infectious orthopedic disease, or noninfectious orthopedic disease. Foals from mares with a normal placenta were more likely to have an initial IgG of >8 g/L than foals from mares with placentitis ($p=0.0002$, RR 5.3, 95% CI 1.4-19.7). Foals from mares with placentitis were more likely to have a low blood glucose ($p=0.0003$, unit OR 1.6) and a high serum creatinine concentration ($p=0.0003$, unit OR 0.99) than foals from mares with normal placentas. The area under the receiver operator characteristic curves for glucose and creatinine were 0.79 and 0.84 respectively, indicating that low blood glucose and high creatinine are good indicators of the presence of placentitis. Foals from mares with placentitis were more likely to have a sepsis score >11, require parenteral nutrition, require inotropes or vasopressors, be recumbent at admission, have poor peripheral pulses and cold extremities than foals from mares with a normal placenta ($p<0.05$). However, foals from mares with placentitis had a similar probability of survival as foals from mares with normal placentas.

These data indicate that placentitis increases the probability of prematurity, dystocia, perinatal asphyxia syndrome and failure of transfer of passive immunity. Foals from mares with placentitis are more likely to present with signs of shock and more likely to require intensive supportive therapy but have the same probability of survival as foals from mares with a normal placenta. The incidence of gross placental pathology in this group of critically ill foals was surprisingly low and further investigation of the risk factors associated with the admission to the ICU is required.

FORM OF ALPHA-TOCOPHEROL AFFECTS VITAMIN E BIOAVAILABILITY IN THOROUGHBRED HORSES

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Vitamin E can be obtained from natural or synthetic sources, but the chemical structure of each is different. The following studies were conducted to determine if synthetic and natural-source vitamin E have similar bioavailabilities when administered at equal IU doses and to determine if water-dispersible forms of vitamin E are more bioavailable than lipid-soluble forms. In study 1, single 5000-IU oral doses of three different forms of vitamin E were evaluated in eight Thoroughbreds during three one-week periods. The forms tested were synthetic vitamin E (dl- α -tocopheryl acetate) (SYN); natural-source vitamin E acetate (d- α -tocopheryl acetate) (ACT); and natural-source alcohol (d- α -tocopherol) (ALC). Baseline blood serum samples were collected immediately before dosing and at 3, 6, 9, 12, and 24 h post-dosing. In study 2, three Thoroughbreds were used in a replicated 3 x 3 Latin square design trial to assess the relative bioavailability of 5000-IU oral doses of synthetic vitamin E (dl- α -tocopheryl acetate) (SYN); a micellized d- α -tocopherol (Elevate WS); and a d- α -tocopherol (Nano·E) that had been nanodispersed into liposomes. Baseline blood serum samples were collected immediately before dosing and at 3, 6, 9, 12, 24, 36, and 48 h post-dosing. In study 1, ACT and ALC had a significantly greater AUC than SYN ($P < 0.05$). There was no significant difference in AUC between ACT and ALC. Relative to SYN, the bioavailability of ACT and ALC equaled 197% and 252%, respectively.

Table 1: Response in serum α -tocopherol to 5000IU doses of a synthetic and two water-dispersible forms of vitamin E.

treatment	Synthetic ^a	Elevate WS ^d	Nano·E ^e
n	6	6	6
area under curve (48 hrs)	15.62 \pm 3.22 ^a	87.36 \pm 25.3 ^b	95.85 \pm 25.7 ^b
baseline vitamin E (ug/ml)	3.04 \pm .30 ^a	3.00 \pm .39 ^a	2.86 \pm .42 ^a
peak vitamin E (ug/ml)	3.63 \pm .36 ^a	6.01 \pm 1.26 ^{ab}	6.69 \pm 1.39 ^b
Δ vitamin E (ug/ml)	.59 \pm .08 ^a	3.00 \pm .89 ^{ab}	3.83 \pm 1.15 ^b

^{ab}Means for the same item with the same letter are not different ($P > 0.05$)

In study 2 (Table 1), Elevate WS and Nano·E had a significantly greater AUC than SYN ($P < 0.05$). There was no significant difference in AUC between Elevate and Nano·E. Relative to SYN, the bioavailability of Elevate WS and Nano·E equaled 559% and 613%, respectively. Nano·E had significantly higher peak and maximal change from baseline values compared to SYN ($P < 0.05$). The results of these studies suggest that natural sources of vitamin E have a greater bioavailability than is accounted for in the current conversion factors of 1.36 and 1.49 used in the feed industry for natural acetate and alcohol, respectively. Natural-source, water-dispersible forms of vitamin E were five to six times more bioavailable than synthetic vitamin E acetate, and a 5000-IU dose more than doubled serum vitamin E levels within 12 h.

VITAMIN K FUNCTION AND BONE DENSITY OF GROWING HORSES

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Vitamin K (VK) has three major forms; phyloquinone (K1) which is synthesized in fresh green plants, menaquinone (K2) which is synthesized by gut bacteria and menadione (K3), the synthetic form which is added to stockfeed. Although usually only considered for its role in blood clotting, VK is now known to have a major role in bone metabolism (Pearson, 2007). Interestingly, the diverse functions of VK are mediated through a single major function as a co-factor for γ -glutamylcarboxylase (GGC). The enzyme carboxylates a number of vitamin K-dependent (VKD) proteins which have a diverse range of biological functions (McCann and Ames, 2009), which are summarized in Table 1.

Table 1: Fuction of Vitamin K dependent proteins

VKD protein	Location of carboxylation	Function
Prothrombin, Factors VII, IX & X	Liver	Coagulation
Proteins C, S, & Z	Liver	Anti-coagulation
MGP	Cartilage, Vascular tissue	-ve regulation of calcification
Osteocalcin	Osteoblasts	ECM bone, glucose homeostasis
Periostin	Periosteum, osteoblasts, remodeling tissues	Fibrillogenesis, wound healing, strain repair (heart, tendon etc)
Gas6 (growth arrest specific)	Smooth muscle, endothelium	Surveillance, apoptosis
Keratoepithelin	Most extra-hepatic tissues	Microtubule stability, -ve regulation of mutations
Proline-rich Gla proteins	CNS, Spinal cord, thyroid	Largely unknown

(ECM = extracellular matrix)

Importantly, the VK molecule has a naphthoquinone nucleus (all) and a prenyl side-chain (K1&K2). The side chain is the site of attachment of the VK transport protein which carries VK to extra-hepatic tissues. K3 (menadione), which is assumed to prevent deficiency in feed supplements, has no side-chain, so functions solely in the liver. Prothrombin time, until recently, has been the only diagnostic test for VK deficiency, so deficiency affecting extra-hepatic tissues/organs has been undetected.

The primary source of VK in the food chain is phyloquinone (K1), a transient instantaneous product of photosynthesis in live green leaves but is destroyed in cut leaves of various grasses by light (Biffin *et al.*, 2008b). VK levels were measured in this study in fresh-cut ryegrass, ryegrass hay after 2 days sun-drying, in hay 2 weeks after shedding, and in ryegrass haylage 2 weeks after packing in sealed bag. Levels were 8.9, 2.3, 1.9 and 7.8 mg/kg DM, respectively. Moreover, VK is poorly absorbed (c. 10%) from the GIT of animals, as it is tightly bound within chloroplasts, and being fat soluble is poorly transported in the gut.

It has been shown (Biffin *et al.*, 2008a) that to achieve 90% carboxylation of osteocalcin, 7 mg VK/ day was required by a 500 kg horse in a bioavailable soluble form (quinaquanone®, QAQ). In a subsequent double-blind trial, bone density development was assessed in 26, 2 year-old Thoroughbred racehorses in the same stable. The treatment group received 7 mg QAQ/day and the control group, a blank powder. Density was assessed as radiographic bone aluminium equivalence (RBAE) on digital Xray images of the third metacarpal bone. The trial continued for 8 months and during that period there was greater increase in bone density in the treatment when compared to the control group (Table 2).

Table 2: Change in bone density of 2year-old Thoroughbreds given a bioactive form of Vitamin K

Mean percentage change in RBAE over time					
Date	12/09/2008	14/10/2008	31/12/2008	2/02/2009	8/05/2009
QAQ	0	1.04±0.31	4.54±0.75	7.68±1.67	9.05±0.91
Control	0	0.35±0.71	2.82±2.97	3.97±2.83	5.02±2.17

Osteoporosis (OP) is common in ageing animals and could also be a component in bone degenerative diseases such as navicular disease, sesamoiditis, and 3rd carpal disease. It has also been recognized following lactation in dairy cows, sows and humans (Pearson, 2007). It occurs reliably in laying hens, despite apparently adequate calcium, trace mineral and vitamin nutrition (Leeson and Summers, 1997). Laying hens therefore can be used as a model to discover whether osteoporosis can be prevented/reversed. The efficacy of bioavailable VK (QAQ) was evaluated in 42 commercial layers, near peak lay (27 wks of age). The hens were divided into 4 treatment groups (Table 3) and Xrayed and RBAE assessed before and after 8 weeks supplementation. The results show an improvement in bone density following supplementation when compared to K3.

Table 3: Bone density response of hens supplemented with bioavailable Vitamin K

Daily dose ug	250 menadione	250 QAQ	500 QAQ	1000 QAQ
RBAE change %	-5.4±1.2	+3.8±2.9	+3.0±2.6	+5.1±3.5

Osteochondrosis (OCD) in pre-sale Thoroughbred yearlings is documented in a radiographic repository and if apparent, has a significant impact on yearling sale price. In this trial, 8 yearlings with severe OCD lesions on Xray were supplemented with 14 mg QAQ/day for 3-7 months and then re-examined. Severity of visible lesions were scored 0-3, 0 = no significant visible lesions, 3 = likely to be career-threatening or requiring surgery and the results in Table 4 show that VK supplementation was associated with a significant reversal in the severity of lesion score.

Table 4: OCD lesion score of 8 Thoroughbred yearlings before and after supplementation with bioavailable Vitamin K

Case	1	2	3	4	5	6	7	8
Initial	3	3	3	3	3	3	3	3
ReXray	1	1	0	1	1	1	2	3

The results of these studies and our previous studies (Biffin *et al.*, 2008a,b) suggest that many horses may receive suboptimal intakes of VK and further research is required to determine equine VK requirements in different management systems and the efficacy of different forms of the vitamin.

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MINERAL VARIABILITY AND HEAVY METAL CONTAMINATION OF CALCIUM CARBONATE AND DICALCIUM PHOSPHATE

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Calcium carbonate (CaCO_3) and dicalcium phosphate (DCP) are used to supply calcium and phosphorus in almost every fortified equine feed available worldwide. The accurate calculation of use rates of these raw materials to achieve the desired level of calcium and phosphorous in feeds and to maintain acceptably low levels of heavy metals requires detailed knowledge of the mineral and heavy metal levels in CaCO_3 and DCP. Because of the common nature of CaCO_3 and DCP, book values or supplier specifications are often relied upon for calcium, phosphorous and heavy metal concentrations. However, testing of CaCO_3 and DCP suggests that this may not be reliable, with mineral and heavy metal values varying widely.

Twenty five samples of DCP and 10 samples of CaCO_3 from batches destined for horse feed manufacture (Pryde's EasiFeed Pty. Ltd., NSW) were collected from December 2008 to April 2010 and analysed (Symbio Alliance, Qld) for calcium, phosphorous, arsenic, mercury and lead.

Table 1: The average \pm SD, highest tested value, lowest tested value and reference value (NRC 2007) for calcium and phosphorous levels in dicalcium phosphate and calcium carbonate.

	Dicalcium Phosphate		Calcium Carbonate
	<i>Calcium</i>	<i>Phosphorous</i>	<i>Calcium</i>
Tested Average (%)	26.4 \pm 4.46	18.8 \pm 1.00	40.5 \pm 1.86
Highest Tested Value (%)	39	19.9	44.2
Lowest Tested Value (%)	20.2	17.8	37.4
NRC Reference Value (%)	22	19.3	39.4

The greatest variation was seen in the calcium level of DCP. Average calcium content was 4.4% higher than the reference value for DCP (NRC 2007), while the highest tested calcium level was 17% higher than the reference value (Table 1). Dicalcium phosphate is also a more heavily contaminated raw material than CaCO_3 , with levels of arsenic and mercury tested at concentrations higher than acceptable for manufacturing equine feeds (Table 2).

Table 2: The average \pm SD, highest tested value, lowest tested value and highest acceptable manufacturing level (Pryde's Pty Ltd) for arsenic, mercury and lead in dicalcium phosphate and calcium carbonate.

	Dicalcium Phosphate			Calcium Carbonate		
	<i>Arsenic</i>	<i>Mercury</i>	<i>Lead</i>	<i>Arsenic</i>	<i>Mercury</i>	<i>Lead</i>
Tested Average (ppm)	11.4 \pm 8.54	0.28 \pm 0.51	10.2 \pm 9.60	2.1 \pm 2.43	0.01 \pm 0.004	1.3 \pm 0.64
Highest Tested Value (ppm)	46 [#]	1.5 [#]	24.6	5	0.02	2
Lowest Tested Value (ppm)	5	0.01	0.08	0.1	0.01	0.28
Highest Acceptable Level (ppm)	20	1.2	50	20	1.2	50

[#] Batches with these concentrations of arsenic and mercury were rejected

The levels of variation and contamination observed within these raw materials illustrates that stringent testing is required to ensure accurate levels of calcium and phosphorous and safe levels of heavy metals in feeds intended for horses.

FATTY ACID SUPPLEMENTS IN THE EQUINE MARKET

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The use of oil-based supplements has increased in the equine sector in recent years – with various claims ranging from the basic ‘shiny coat’ claims to more complex immune function parameters, as well as potential for behavioural and gut-level benefits. The supplements range widely in the types of oil, or blends, included, quality, stability and shelf life and potential technical benefits. For example, lecithins isolate from soya have been shown to reduce excitable behaviour. Many joint formulae contain oils associated with reducing inflammation, although this is a topic much debated in the literature. From a commercial point of view, horse owners are familiar with seeing supplements and products promoting ‘omega oils’ or ‘omega fatty acids’, and these are increasing in popularity and variation, although some appear more effective than others, and there are products that lack scientific proof of the claims they make on packaging. Fatty acids are the basic units within fats and oils. Mammals are unable to make certain types of fatty acids, which are known as ‘essential’ fatty acids, and deficiencies are related to dermatitis, poor fertility, undeveloped brain and retinal function (especially in young stock), stunted growth and poor inflammatory responses. There is a growing level of information available for the ω -3 and 6 oils, and increasing scientific interest in ω -7 and 9.

ω -3 fatty acids are found in fish oils and seed, berry or bean-derived oils, including linseed, rapeseed and soy oil, and in milk derivatives, such as whey or dried milk powder. This type of fatty acid is required for immune response regulation, controlling inflammation. Fish oil is useful for inflammatory problems such as arthritis or dermatitis (sweet itch), regulating blood pressure and clotting time, red blood cell production, hormone production, preventing renal disease and inhibiting tumour growth. Horses who have an *E. coli* infection in their gut often have a major reaction to the endotoxins this bacteria produce, and ω -3 fatty acids have been reported to reduce the severity of these symptoms.

ω -6 fatty acids are specifically associated with the construction and maintenance of liver cell membranes and platelet production. Many oils, including flaxseed, sunflower and safflower contain both ω 3 and 6 fatty acids. ω -9 fatty acids are needed for membranes and hormone production. Fatty acids from animal sources, palm oil, olive oil or blended vegetable oils tend to be low in ω -6 fatty acids. Some specific benefits for oils have been shown in clinical studies. For example, sunflower oil is reported to benefit certain immune cells and reduces pulmonary inflammation in horses with airway obstruction disorders (Khol-Parisini *et al.*, 2007).

Certain berries contain ω -3, 6, 7 and 9 fatty acids, along with fat-soluble vitamins and natural background antioxidants. Trials on these plant oil extracts have been shown to provide all the benefits associated with essential fatty acid supplementation, plus being afforded the protection of the antioxidant compounds.

The balance of ω -6 to 3 fatty acids is important, and care should be taken not to intake too much ω -6, although this recommendation is derived from human research. Currently, in humans, ratios of 5:1 are recommended (so there is more ω -3 than ω -6 present), although the optimal ratio for horses has yet to be agreed upon.

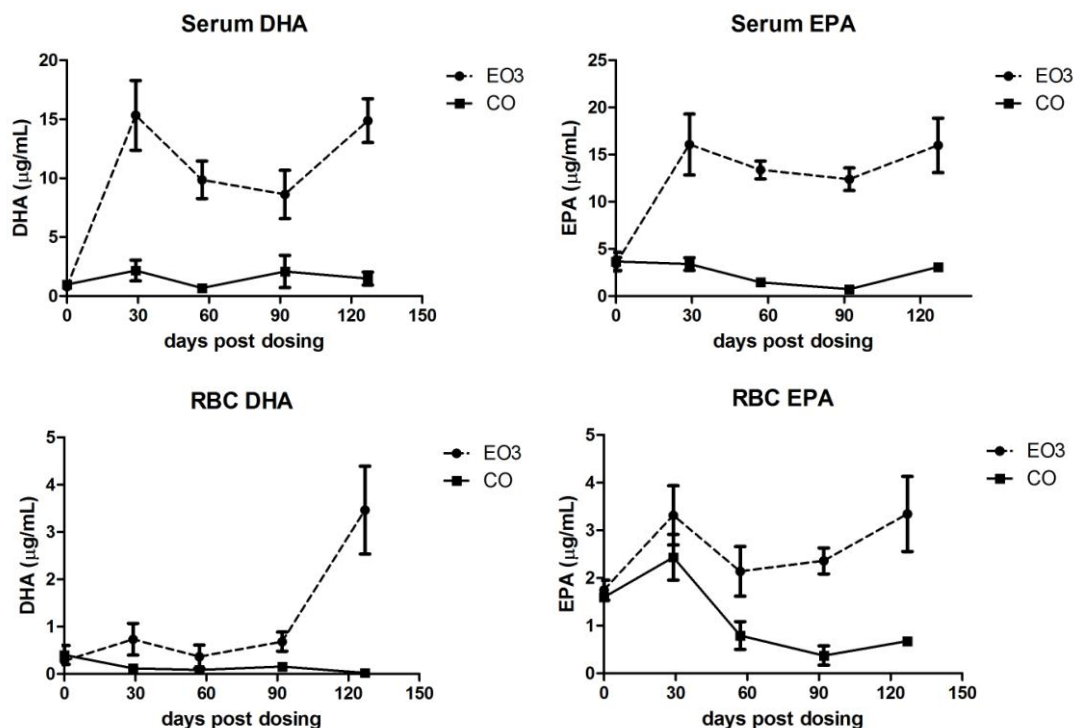
FISH OIL AND CORN OIL SUPPLEMENTATION AFFECT RED BLOOD CELL AND SERUM EICOSAPENTAENOIC ACID (EPA) AND DOCOSAHEXAENOIC ACID (DHA) CONCENTRATIONS IN THOROUGHBRED HORSES

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Horses require both omega-3 and omega-6 fatty acids in their diets. The omega-3 family stems from alpha-linolenic acid (ALA), while the omega-6 family originates from linoleic acid (LA). Long-chain, omega-3 fatty acids eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are intermediates in the formation of eicosanoids that have been shown to reduce inflammatory responses, support immune function, and enhance fertility. This study was conducted to compare the effect of supplementation with oil high in EPA and DHA (fish oil) or low in EPA and DHA (corn oil) on red blood cell (RBC) and serum EPA and DHA. Twelve Thoroughbred geldings were supplemented for 127 d with 60 ml of either fish oil (EO-3TM) or corn oil. Blood samples were taken at d 0, 29, 57, 92, and 127 and analyzed for EPA and DHA. By d 29, horses receiving fish oil had an average increase in serum EPA and DHA of 3.7-fold ($P \leq 0.05$) and 17.9-fold ($P \leq 0.01$), respectively. In horses receiving corn oil, serum EPA decreased 1.5-fold from baseline at d 57 ($P \leq 0.05$) and fourfold by d 92 ($P \leq 0.05$). By d 127, RBC DHA concentrations in the fish oil supplemented horses was over 1.9-fold greater ($P \leq 0.05$) than baseline, while there was no significant difference observed in RBC DHA from horses receiving corn oil. In the fish oil supplemented group, RBC EPA increased 11.5-fold ($P \leq 0.05$) by d 127. Corn oil supplemented horses had significantly lower than baseline RBC EPA at 57 d ($P \leq 0.05$), 92 d, and 127 d ($P \leq 0.01$). This study showed that 60 ml/d of fish oil supplementation significantly increases both serum and RBC EPA and DHA in horses. Corn oil supplementation resulted in a significant decrease in RBC EPA, which may affect RBC membrane fragility.



INFLUENCE OF A CANOLA-BASED OMEGA OILS SOURCE ON IMMUNITY IN MATURE HORSES

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Bioactive molecules can influence a number of functions within the animal, including immunity. There is currently very little scientific information available in the literature on the immune enhancing effects of nutraceutical ingredients in horses. Investigations into the effects of omega-3 and omega-6 fatty acids on immune function, inflammatory responses, and lipid peroxidation have been carried out in a number of species including dogs, rats, and humans. However, in mammalian studies, the same ratios of omega-3: omega-6 fatty acids have produced conflicting results. Some trials have shown enhancement of immune responses such as T and B cell mitogenic responses, whereas in others, suppression of cell-mediated immune responses were reported. These differences may be due to the way the oils are metabolized and utilized in the gut and cells. Little research investigating the immune effects of dietary supplementation with omega-3 and omega-6 fatty acids appears to have been carried out in horses.

Sixteen adult mixed breed riding horses were housed in 4m x 3.6m outdoor pens with peeled wood bedding and *ad lib* access to water. They were fed a balanced hard feed (NRC, 2007) and hay, according to body weight. The horses were randomly allocated to two treatments; 1. test diet (OM) containing 60ml/d of canola-based oil supplement (VitaPower Ltd., Wanganui) 2. control diet (CO) containing 60 ml/d of iso-energetic oil (Coconut Oil, Davis Trading Ltd., Palmerston North). A range of immune parameters, including: lymphocyte proliferation, phagocytic activity, specific immunoglobulin levels, and the expression of a number of cell surface markers e.g. T helper cell, cytotoxic T-cell markers were measured following a tetanus vaccine challenge (Equivac-T) on d1. The horses received the supplement for a period of 28 days, with immune testing being carried out prior to dietary supplementation and vaccine challenge and after 14 d and 28 d of feeding. Previous studies in cats and dogs have shown that a similar product significantly enhanced different aspects of immunity over a similar four week period (unpublished data). Results between treatments were compared over time. Analysis of variance (ANOVA, SAS, 1999) was used to compare differences based on 5% confidence limits.

There was no significant change in lymphocyte proliferative responses over the trial period for either diet. A slight increase in phagocytic activity in the OM-fed animals (d 14: 16.4% vs. 18.8%; d 28 16.6% vs. 18.9% for CO and OM respectively) was recorded, but was not significantly different to baseline levels measured on d1. Dietary supplementation with both oils caused a significant increase in specific IgG levels 14 d after vaccination, which continued to the end of the trial period. The control group showed a highly significant ($P<0.001$) increase in IgG titres after 14 d (1356 mg/ml) and 28 d (940 mg/ml) compared to pre-vaccination levels (100 mg/ml at d 1). The test group showed a similar significant ($P<0.01$) change in serum IgG levels over the same time period (70 mg/ml d 1; 561 mg/ml d 14; 369 mg/ml d 28), but this was a significantly lower IgG response ($P<0.01$) compared to the CO group. The dampening down of the IgG response in horses fed OM may provide insight to the observed improvement in performance of horses receiving the product in the field. Work in other species (Mooney *et al.*, 1998; Kearns *et al.*, 1999) suggests that omega-3 fatty acids act as substrates for eicosanoid metabolism, resulting in the production of eicosanoids with a lower inflammatory potential than those produced from omega-6 fatty acids. The reduction of levels of inflammation in the horse can be important in reducing inflammation through injury or disease, and faster recovery following vaccination challenge.

INFLUENCE OF A COMMERCIAL OMEGA OILS SOURCE ON DIGESTIBILITY AND BODY CONDITION IN MATURE HORSES

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Although many commercial claims have been made, there is little actual scientific information available on the benefits of specialist oil supplements in horses. The potential benefits of dietary supplementation with omega-3 fatty acids (alpha-linolenic acid) and the ratio of omega-3 to omega-6 fatty acids (linoleic acid), which are found in high quantities in canola oil, makes it interesting as a functional supplement for horses. Previous studies in cats and dogs showed that canola-based supplements significantly improved the digestibility of the diet when fed over 4 week period (Rutherford-Markwick et al., 2008).

Sixteen adult mixed breed riding horses were housed in 4m x 3.6m outdoor pens with peeled wood bedding with *ad lib* access to water. They were fed a balanced hard feed (NRC, 2007) and hay, according to body weight. The horses were randomly allocated to two treatments; 1. test diet (OM) containing 60mls/d of canola-based omega oil supplement or 2. control diet (CO) containing 60 ml/d of iso-energetic oil (commercial coconut oil). Digestibility, faecal quality and body condition were monitored throughout the 28d trial. Faecal quality, body weight (by girth tape) and body condition were monitored on days 1, 14 and 28. Horse were visually condition scored on a five point scale, (1 = emaciated, 3 = optimum and 5 = obese). Faecal quality was visually assessed using a 1 to 5 scale (1 = diarrhoea, 5 = well-formed pellets). Faeces were collected over a 12 hour period on days 26, 27 and 28 and pooled for analysis. Dry matter digestibility was measured by the forage ash method (Miraglia et al., 1999). Results between treatments were compared over time. Analysis of variance (ANOVA, SAS, 1999) was used to compare differences based on 5% confidence limits.

There were no differences in bodyweight between treatments over the four week period ($P>0.05$). Despite the similarity in bodyweight, there was a numeric difference in initial body condition scores (CO: 2.81 vs. OM: 2.31; $P>0.05$). During the trial, horses fed OM showed a greater improvement in condition compared to CO (21.6% vs 8.9% week 2; 28.4% vs 14.4% week 4, for OM versus CO respectively). For faecal quality, horses had a 14.3% vs. 13.8% improvement at 14d, and a 42.9% vs. 37.9% improvement after 28d for OM versus CO respectively. The OM diet showed a strong trend ($P=0.07$) for improved digestibility, with 41% vs. 35% for the CO diet.

Feeding OM gave a greater improvement in both condition scores and faecal scores during the study, although these were not significant. The 6% improvement in digestibility appeared to be attributable to the fatty acid profile of the supplement and its high unsaturated fat content.

A CROSS SECTIONAL ANALYSIS OF BREEDING PATTERNS IN THE NEW ZEALAND THOROUGHBRED BROODMARE HERD

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The Thoroughbred stud book is the largest and most commercially orientated studbook in New Zealand, with approximately 40% of the foal crop exported each year. Over the last 2 decades there has been a dramatic decrease in the size of the active broodmare herd from 10176 mares in 1989 to 6488 mares in 2004. It has been proposed that the reduction in the broodmare herd has been achieved by the removal of the non-commercial breeding stock.

The aim of this paper was to examine the relative contribution of sires in 4 different categories, namely Low cost (<\$5000), Medium (\$5001-\$10,000), Expensive (>\$10,000) and Shuttle on the reproductive success of their progeny, and the rate and retention of these sires within the New Zealand Thoroughbred broodmare herd.

In the 1998/99 breeding season 146 sires covered 6768 mares of which the majority were covered by Low cost sires (63%), Medium (19%) and Expensive (6%) and Shuttle sires (12%). The foal crop was skewed towards foals by Low cost (n=1916, 44%) and Medium cost sires (n=1024, 24%). Expensive and Shuttle sires were responsible for 520 (12%) and 891 (20%) of the foals.

Using a subset of 12 sires (3 random sires from each sire category), and the resultant filly foal population, it was identified that a greater proportion of the filly foals by Expensive and Shuttle sires were offered for sale as yearlings (37% and 34%, $p=0.001$, respectively) or exported (31% and 26%, $p=0.001$, respectively) than other sire categories. Filly foals by Expensive and Shuttle sires were also more likely to have a race preparation (83/77 (85%) & 44/54 (82%)) than fillies by Medium and Low cost sires (45/70 (64%) & 12/24 (50%), $p=0.001$).

The majority of the fillies by Expensive sires entered the breeding population (80%) compared to less than half the fillies by Shuttle (49%), Medium (43%) or Low cost sires (25%) ($p=0.001$). The fillies by Low cost sires entered breeding later (median 8 years old, 95%CI 6.8-9.1) than Medium (5 yrs 95% CI 4.3-5.6) or Shuttle and Expensive (6 years old 95%CI 5.5-6.4). The fillies by Low cost sires were also of lower parity (2, 95%CI 0.4-3.6) than Medium (3, 95%CI 1.27-4.47), Expensive (4, 95%CI 2.9-5.0) or Shuttle (5, 95%CI 2.8-7.1) sire categories.

The majority of the fillies that had bred had raced (81%). Whereas retrospective examination of the dams and maternal granddams of these filly foals identified 69% of their dams and only 45% of the maternal granddams had raced. There was no inter-generation effect on the age of entering the breeding population, though maternal granddams appeared to be of lower parity (2, 95%CI 1.5-2.5) than dams or fillies (4, 95%CI, 3-4.9) $p=0.008$.

There was moderate bias of the dams sire category influencing sire category of the filly. However, Expensive sires covered some mares by Low cost sires and vice versa. While Low cost sires contributed the greatest number of foals in the population few appear to breed on (8% of fillies that bred were by Low cost sires) and this trend was consistent for the dams (10%) and granddams (4%).

The data indicates that filly foals by Expensive and Shuttle sires have a greater opportunity race and enter the breeding population. Filly foals by Low cost sires must race to demonstrate genetic merit and so enter the breeding herd later. Perhaps in line with more commercial focus of breeding stock, fillies are more likely to have been raced than their granddams.

LACTATING, MULTIPAROUS MARES HAVE HIGHER OESTRADIOL 17- β LEVELS IN EARLY PREGNANCY THAN MAIDEN MARES

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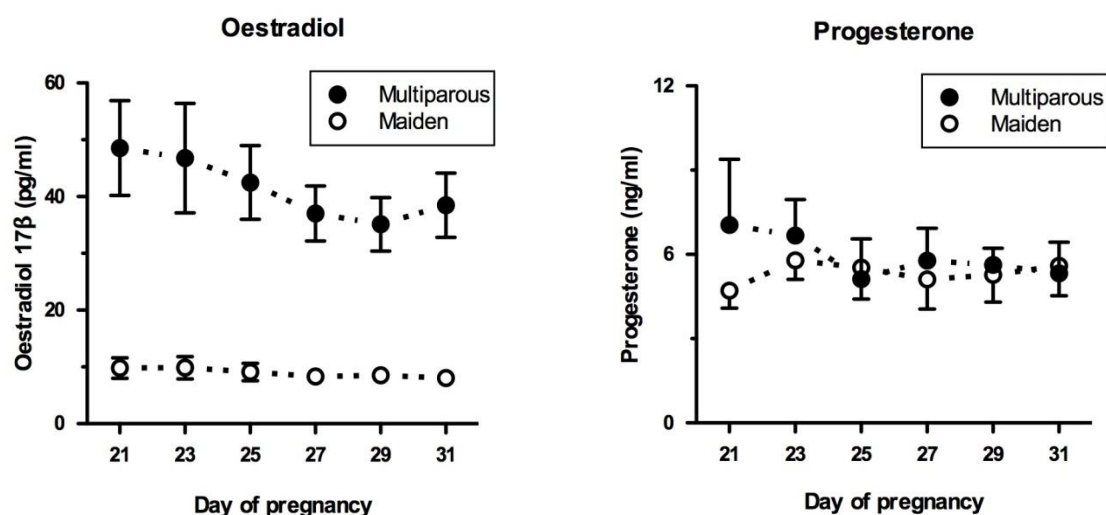
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The primary corpus luteum (CL) is the major source of progestagens for the first 40-50 days of pregnancy. However, the primary CL also produces oestrogens, as it expresses cytochrome P450 aromatase. Further the equine embryo itself is capable of oestrogen synthesis in early pregnancy and this appears to be important in the establishment of pregnancy. Despite these multiple sources of oestrogen all previous studies show that circulating oestrogen levels in early pregnancy are similar to those observed in dioestrus, and only increase markedly in conjunction with the onset of equine chorionic gonadotrophin (eCG) secretion around day 35 of pregnancy.

While investigating the role of processionary caterpillars in the aetiology of equine amnionitis and foetal loss (Cawdell-Smith et al. 2010) steroid levels were measured in lactating, multiparous mares (n=4) and maiden mares (n=7) during early pregnancy. Blood samples were obtained each morning from the jugular vein and plasma was stored at -20°C. Plasma progesterone and oestradiol 17 β were both measured by RIA. Mares were of mixed breeds (Standardbred and Australian Stockhorse), aged 3-11 years.

Figure 1. Mean plasma steroid concentrations (\pm SEM) of mares in early pregnancy



No differences in plasma progesterone concentrations were observed between lactating, multiparous mares and maiden mares, but oestradiol concentrations were approximately 4-fold higher in lactating, multiparous mares (Figure 1). Earlier reports note only low oestrogen levels prior to eCG stimulation, but these studies measured either total oestrogens or oestrone sulfate, and make no reference to mare status. Interestingly, Allen et al. (1995) showed that oestradiol levels are significantly higher in lactating mares compared to maiden and barren mares around the time of ovulation. Further, Papa et al. (1998) found low oestradiol levels, rather than progesterone, are characteristic of mares with early embryonic loss. These reports together with our own unexpected observations suggest that closer examination of oestradiol 17 β levels in early pregnancy of the mare is required.

Cawdell-Smith A.J. et al. 2010 *Anim. Reprod. Sci.* (in press)

Allen W.R. et al. (1995) *Equine Vet. J.* 27, 460-464

Papa F.O. et al. (1998) *Braz. J. Vet. Res. Anim. Sci.* 35, 170-173

GLUCOSE AND INSULIN DYNAMICS IN GESTATING MARES AND FOALS

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Pregnancy is characterized by a progressive decline in insulin sensitivity, a natural adaptation that parallels growth of the feto-placental unit ensuring sufficient glucose supply to the fetus. Dietary energy composition, specifically feeds high in non-structural carbohydrates, are known to diminish insulin sensitivity, increasing risk for diseases such as obesity and laminitis in the horse. As the fetus is glucose dependent, gestation is of period that can be greatly influenced by the environment, including maternal nutrition. Thus, mare maternal nutrition may perturb glucose and insulin dynamics during gestation, causing implications in the resulting neonatal foal, potentially predisposing it to metabolic disorders associated with insulin resistance later in life.

The current study investigates the relationship between glucose and insulin dynamics in the gestating mare and that of her subsequent foal. Pregnant (n=12) and non-pregnant (n=6) mares were used in the study and offered either a high (n=6) or low carbohydrate (n=6) diet during the last trimester of pregnancy. Specific basal proxies (Treiber *et al.*, 2005) were used to determine mare's monthly metabolic status (insulin sensitivity) in conjunction with body condition score, body weight and detailed nutritional intake. The insulin-modified frequently sampled intravenous glucose tolerance tests (FSIGT) were applied to pregnant mares at 71 +/- 2 days of gestation, and 4 +/- 1 days prior to parturition. Non-pregnant mares were tested at the same time. Neonatal foal FSIGTs (n = 11) were conducted at 14 +/- 2 days of age. The minimal model of glucose and insulin dynamics was used to determine insulin sensitivity (SI), glucose effectiveness (Sg), acute insulin response to glucose (AIRg) and disposition index (DI). This study is ongoing and will offer new data examining glucose and insulin dynamics in mares throughout gestation and the relationship between maternal nutrition and neonatal development.

Treiber, K.H., Kronfeld, D.S., Hess, T.M., Boston, R.C. and Harris, P.A. (2005) Use of proxies and reference quintiles obtained from minimal model analysis for determination of insulin sensitivity and pancreatic beta-cell responsiveness in horses. *American Journal of Veterinary Research* 66, 2114-2121.

STORAGE OF STALLION SEMEN AT AMBIENT TEMPERATURE

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Artificial insemination (AI) is now common practice in the equine breeding industry and is accepted by the majority of breed societies. All three forms of artificially collected semen, fresh, fresh-chilled or frozen, can be used for equine AI. Frozen semen enables flexibility for transport and long-term storage. However, the conception rates associated with the use of frozen semen are variable and considerably lower than those for fresh and fresh-chilled semen. Fresh-chilled equine semen at 5°C only remains viable for a relatively short period. The objective of this study was to develop a semen extender that is able to maintain the viability of spermatozoa for more than the current standard of 48 hrs and be stored at ambient temperature (approximately 17 - 20°C), thus permitting greater ease of transport whilst maintaining acceptable levels of conception rates which are close to those for fresh semen. The project investigated a bovine extender designed for storage of semen at ambient temperature to determine whether it would prolong the viability of stallion spermatozoa when maintained at ambient temperatures.

Semen samples from three stallions were collected, assessed and extended with four different extenders. Three of the four extenders are commercially available and one experimental extender was examined. The extended samples were stored at both 17°C and 5°C. Ejaculates were evaluated at the time of collection, and then every 24hrs for seven days. Semen samples were evaluated for % motile, rate of motility, % live, % intact acrosome and chromatin integrity. The experimental extender maintained sperm motility above 45% at 5°C and for up to 96 hrs and 120 hrs respectively. This result was a significant improvement in storage time compared to commercial extenders ($P<0.05$). Similarly, the experimental extender maintained the rate of motility above level of 2 for a longer storage period than commercial extenders. The percentage of acrosome intact spermatozoa significantly decreased ($P<0.05$) for the test extender at both 5°C and 17°C. However, spermatozoa in the experimental extender at 5°C remained above the threshold level of 30% for up to 144 hrs, although at 17 °C, maintenance of intact spermatozoa was reduced to 24 hrs. Neither % live nor % DNA denaturation was significantly affected between the extenders.

In summary, the results of this study has shown that the experimental extender has the potential to maintain viability of stallion semen for over 96 hrs at 5°C, which is an 48 hrs longer than the current recommendation time for fresh-chilled equine semen. This extender may be an option for use at 5°C if adjustments are made to the total number of spermatozoa that are shipped as an insemination dose. This would compensate for the greater number of sperm that undergo the acrosome reaction in this extender. Further studies are required to investigate the fertilizing capacity of the spermatozoa extended with the experimental extender used in this current study. Identification of the cause for an increased % acrosome reacted spermatozoa should also be investigated when stored at ambient temperatures in order to improve this extender for use at ambient temperatures.

This research was supported by the RIRDC Horse Program

FEEDING TO MAINTAIN INSULIN SENSITIVITY

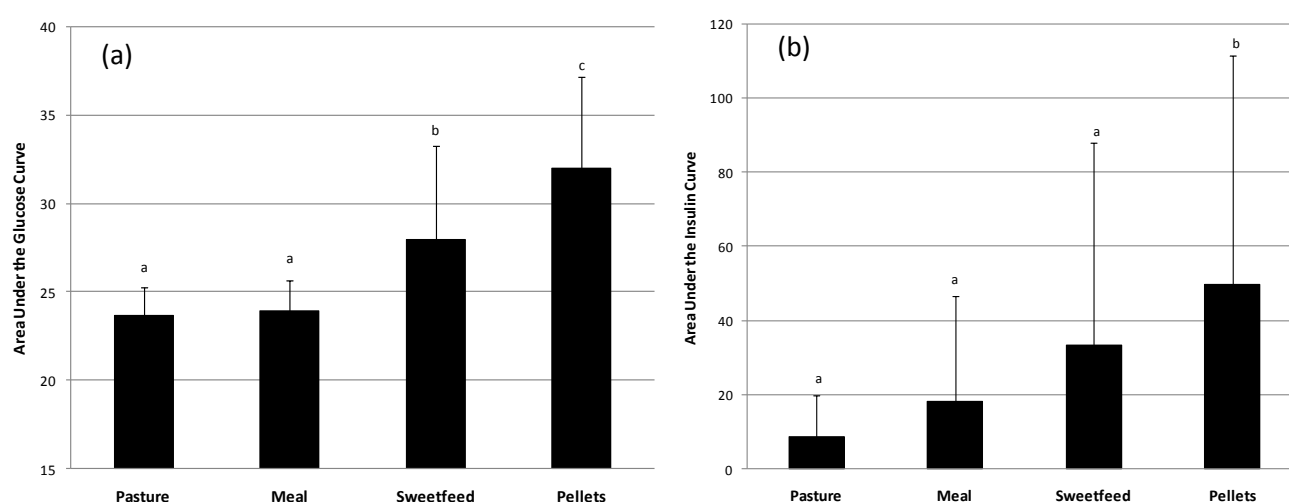
N. Richards

Equilize Horse Nutrition Pty Ltd, Newcastle, NSW

While obesity and inactivity are almost certainly implicated in insulin resistance, diet, and specifically the consumption of high non-structural carbohydrate (NSC) feeds appear to negatively impact a horse's insulin sensitivity (Treiber *et al* 2005; Quinn *et al* 2008). Feeding a low NSC diet therefore seems to be a sensible recommendation for horse owners wishing to maintain long-term insulin sensitivity. The aim of the study was to add to the current data available to help identify the level of NSC least likely to impact post-feeding glucose and insulin responses in horses and therefore more likely to maintain long term insulin sensitivity.

Four horses were fed four diets; 24 hour pasture access (Pasture) with 7% DM NSC; or 10 hours pasture access supplemented with either 1% bodyweight of copra meal (Meal) with 11% DM NSC; extruded and pelleted feed (Pellet) with 25.3% DM NSC; or sweetfeed (Sweetfeed) with 33.7% DM NSC. Supplementary feeds were divided and fed in 2 meals per day. Horses were adapted to diets for 4 days. On the morning of the 5th day, a pre-feeding blood sample was taken. Horses were fed and blood samples were collected over a 6 hour period for a total of 13 samples per horse including the pre-feeding sample. Blood was immediately centrifuged and the plasma frozen until analysis. All samples were analysed for plasma glucose and insulin. Results were statistically analysed using a restricted maximum likelihood (REML) procedure to assess the effects of diet, period, and time. Significance of terms was assessed using Wald chi-square and *F* tests.

Figure 1: The mean area \pm s.e. under the Glucose (a) and Insulin (b) response curves.



Diet had a significant effect on the area under the glucose ($P < 0.007$) and insulin ($P < 0.001$) response curves. The copra meal diet at 11% NSC did not differ significantly from the 7% NSC pasture, suggesting that feeds with a dry matter NSC level of 11% or less may be useful as a supplementary energy source where maintaining insulin sensitivity is a priority.

Treiber, K.H. *et al* (2005). *Journal of Animal Science* 83, 2357–2364.

Quinn, W. *et al* (2008). *Journal of Equine Veterinary Science* 28, 728 – 738.

THE EFFECT OF SHORT TERM ADAPTATION TO A HIGH FAT DIET ON INSULIN SENSITIVITY IN AGED THOROUGHBRED HORSES

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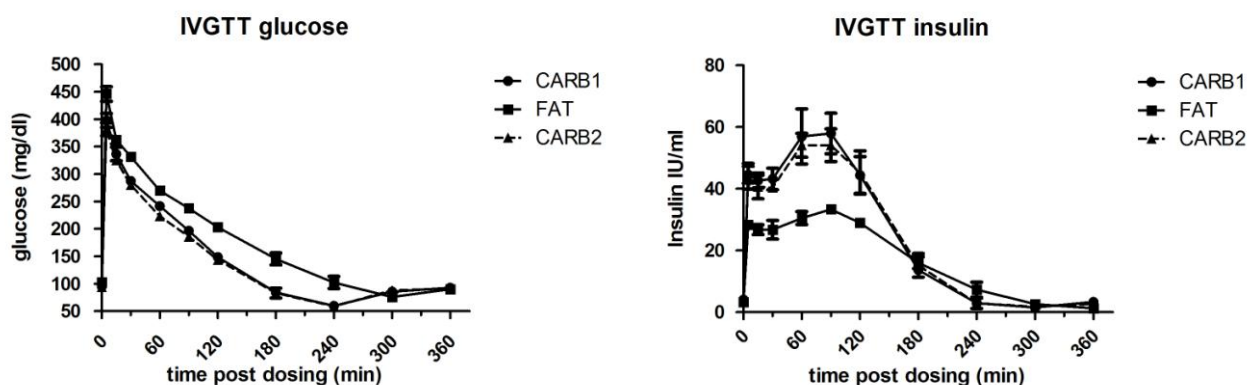
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Insulin resistance is an associated risk factor in laminitis, Equine metabolic syndrome and Equine Cushing's Disease. Aged horses are at particular risk of developing these diseases. Diets high in non-structural carbohydrate have been reported to reduce insulin sensitivity (SI) in horses, while it has been suggested that high fat diets may modify SI. In man, high fat diets reduce SI and some equine studies have shown an increase, whilst others showed reduced SI. The objective of this study was to examine whether short term adaptation to a high fat diet would affect insulin sensitivity in aged horses. Three Thoroughbred geldings (18-24 yr; BCS 6.5 - 7; weight 591.0 ± 51.0 kg) were used in a three period longitudinal study with each period lasting 35 days. During the first and third periods, the horses were fed 9.09 ± 1.90 kg/d of mixed grass/legume hay along with 2.5 kg of an unfortified sweet feed (CHO 1 and 2).

During period 2 the horses received the same amount of mixed hay along with 1.5 kg of a grass/legume hay cube and 600 ml of soybean oil (FAT). An oral (OGTT) and intravenous (IVGTT) glucose tolerance test was conducted each period on d 28 and d 35, respectively. In the OGTT a 50% dextrose solution was administered at a rate of 1g glucose/kg BW via nasogastric tube. Blood samples were taken via jugular catheter immediately before (0 min) and at 30, 60, 90, 120, 180, 240, 300 and 360 min post administration. In the IVGTT a 50% dextrose solution was administered intravenously at a rate of 0.5g glucose/kg BW over 10 min and blood samples were collected immediately before (0 min) and at 5, 15, 30, 60, 90, 120, 180, 240, 300 and 360 min post administration. Blood samples taken during the OGTT and IVGTT were analyzed for serum insulin, glucose and non-esterified fatty acids (NEFA).

During the OGTT, glucose was significantly higher in the FAT group at 120 min ($p < .05$) compared to CHO1 and was significantly higher at 120 min ($p < .01$) and 180 min compared to CHO2. Insulin was not significantly different during the OGTT between groups. During the IVGTT, the area under the curve (AUC) for glucose concentration vs time was significantly higher for the FAT group compared to both CHO1 ($p < .01$) and CHO2 ($p < .001$). Glucose during the IVGTT was significantly higher ($p < .05$) in the FAT group at 5, 30, 90, 120, 180, and 240 min compared to CHO1 and at 5, 15, 30, 60, 90, 120, 180, and 240 min compared to CHO2. The AUC for insulin during the IVGTT was not significantly different between treatments, but insulin in the FAT group was significantly lower ($p < .05$) at 5, 15, 30, 60, 90, and 120 min compared to CHO1 and at 5, 60, 90, and 120 min compared to CHO2. The results of this study suggest that feeding high fat to aged horses reduces insulin sensitivity compared to a moderate high carbohydrate diet. During the IVGTT, horses on the high fat diet produced less insulin and took longer to clear glucose from their blood. Further research is needed to determine if these differences were due to high fat in the ration, or a lack of readily digestible carbohydrate.



GLYCEMIC/INSULINAEMIC RESPONSE TO FEEDING HAY WITH HIGH AND LOW NONSTRUCTURAL CARBOHYDRATE CONTENT – THE EFFECT OF BREED AND PSSM STATUS.

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A mutation recently identified in the Glycogen Synthase I gene (*GYS1*) is responsible for Polysaccharide Storage Myopathy (PSSM) type 1. Persistent activation of glycogen synthase enzyme, and increased storage of glycogen and an abnormal polysaccharide is the result. Clinical signs of PSSM include exercise intolerance and muscle pain that increases when horses are fed concentrates high in non-structural carbohydrate (NSC), which induce a high glycemic response. Feeding a low NSC concentrate and providing dietary fat eliminates signs of rhabdomyolysis in 75% and reduces muscle pain in 100% of PSSM horses. Hay is a potential source of NSC in a horse's diet; however, little research quantifies the glycemic response to hay of variable NSC content.

Six healthy control (C) horses and 7 PSSM horses of Quarter Horse related breeds were used to test the glycemic and insulinaemic (G/I) response to hay of varying NSC content. Horses had no access to grain or pasture for 1 month before the study. They were fed high carbohydrate (HC; 17% WSC) or low carbohydrate (LC; 4% WSC) grass hay for 5d in a crossover study with a 7d washout period where medium carbohydrate hay was fed (MC; 11% WSC). Starch content was less than 1.5% in all hays and fructan levels were less than 3.5% whilst protein content was highest in the HC hay. On the last day of each period horses were fed 0.5% BW of hay and time to consumption measured. Blood samples were taken every 30 min for 5 hr to measure glucose by glucometer and insulin by RIA. Area under the curve (AUC) and peak values were compared by 2 way ANOVA with significance set at $p < 0.05$.

Time to consumption varied and was fastest for the HC hay. PSSM horses ate more slowly than control QHs. The glucose response of C horses was similar across hay types, however, a higher insulin response was found on HC vs MC & LC hay. PSSM horses had higher glucose and insulin response to the HC hay than MC & LC hay, but their glucose response was higher and insulin response lower on HC hay than C horses. No difference was found in other hays.

In conclusion, significant differences exist in the G/I response of horses to hay. HC hay led to a higher insulin response than MC and LC hay. PSSM affected the G/I response with PSSM QH showing a lower insulin response to HC than healthy QH which is consistent with higher insulin sensitivity in these horses. These findings were likely influenced by both the NSC content of hay and the rate of intake. Higher NSC content will lead to higher palatability and more rapid intake of HC hays, which can increase glucose absorption and insulin levels in serum after feeding. But factors other than glucose are also involved in the insulin response to feeding.

For both PSSM horses and horses prone to develop conditions related to insulin resistance it may be prudent to avoid feeding hay with an NSC content $>17\%$. Hay with an NSC content of $<10.8\%$ does not appear to influence the G/I response of PSSM or control QH. Blood glucose is not a good indicator of an insulin response to a feed.

PSYLLIUM LOWERS BLOOD GLUCOSE AND INSULIN CONCENTRATIONS IN HORSES

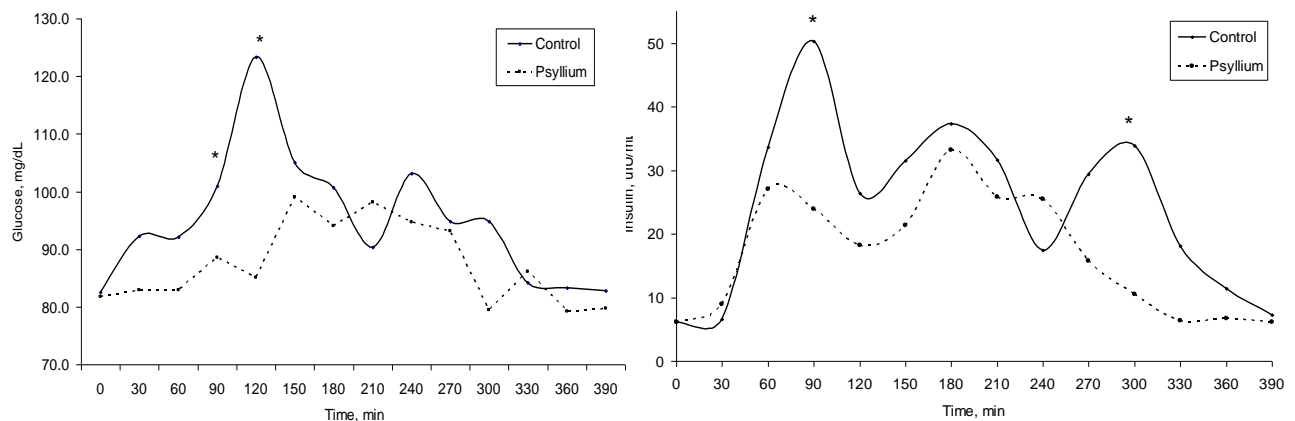
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Psyllium is a dietary fiber that is used in humans to help manage blood glucose and insulin. Metabolic effects of feeding psyllium daily to horses are unknown. Managing blood glucose and insulin in horses has become a major focus of equine research because insulin resistance and the subsequent hyperinsulinemia have been implicated in the pathogenesis of laminitis. Sixteen mature light breed stock horses were used in a completely randomized design to determine how psyllium affects postprandial blood glucose and insulin concentrations. Psyllium was fed with a twice daily grain and hay ration for 60 days. Psyllium treatment levels were: 1) 90 g/d; 2) 180 g/d; 3) 270 g/d; 4) an isocaloric control. Blood was sampled through an intra-jugular catheter every 30 minutes on day 60 starting just prior to morning feeding.

Glucose was lower 90 min (88.6 ± 7.6 versus 101.0 ± 22 mg/dL; $P = 0.05$) and 120 min (85.1 ± 5.1 versus 123.4 ± 21 mg/dL; $P < 0.001$) after feeding in horses on a psyllium treatment compared to those fed an isocaloric control (Figure 1). Insulin concentrations after feeding were lower 90 min (23.9 ± 13.5 versus 50.3 ± 12.8 μ IU/mL; $P = 0.002$) and 300 min (10.5 ± 0.7 versus 33.9 ± 13.5 μ IU/mL; $P < 0.001$) in horses fed psyllium compared to those that were fed an isocaloric control (Figure 2). Psyllium caused a similar response in blood glucose ($P = 0.48$) and insulin ($P = 0.15$) regardless of treatment level (90, 180, and 270g/d).

Figures 1 and 2. Mean blood glucose and insulin of horses supplemented with psyllium (dotted line) or fed an isocaloric control (solid line) during an oral carbohydrate challenge



Psyllium fed daily prevented postprandial hyperglycemia and hyperinsulinemia in this group of normal, non-obese and unexercised horses. Psyllium could be especially beneficial to insulin resistant horses or horses that are predisposed to developing laminitis. Psyllium is commercially marketed and readily available to horse owners, and when fed daily maintained lower blood glucose and insulin concentrations in mature horses.

EQUINE INSULIN DYNAMICS AND DIETARY PROTEIN

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Insulin resistance is becoming a more prevalent problem in the world horse population and is associated with poor athletic and reproductive performance. Performance horses which require increased energy intake usually achieved by increased feed intake are found to receive protein levels which exceed the National Research Councils recommendations. The objective of this study was to evaluate the effects of high and low dietary protein contents on glucose and insulin dynamics in horses using the modified frequently sampled intravenous glucose tolerance test (M-FSIGT) protocol and minimal model analysis.

Twelve mature Standardbred geldings of a similar weight and body condition score were subjected to two M-FSIGTs. The first following the feeding of a hay based basal diet (12days) and the second following the adaptation period (6weeks) to either the high or low protein diets which the horses were allocated to randomly. The horses received the high or low protein diets from the morning following their first M-FSIGT till the afternoon prior to the second M-FSIGT. On the morning of the test the horses received a hay meal and were provided hay ad lib throughout the test period. Catheters were put in place forty five minutes to one hour prior to collection of the baseline sample. A glucose bolus (0.3g/kgBW) was administered and followed by collection of blood samples at 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 14, 16, 19 minutes. At 20minutes an insulin bolus (20mIU/kgBW) was administered and followed by blood samples collected at 22, 23, 24, 25, 27, 30, 35, 40, 50, 60, 70, 80, 90, 100, 120, 150, 180, 210, 240 minutes.

Plasma glucose was analysed commercially using the enzymatic UV test (hexokinase method, Olympus kit on a Olympus AU 400 Analyser, Diagnostics Systems Division, Melville NY) and the plasma insulin was analysed using a commercial radioimmunoassay (Coat A Count Insulin, Siemens Medical Solutions Diagnostics). This data was then used to generate insulin sensitivity (Si), glucose effectiveness (Sg), acute insulin response to glucose (AIRg) and disposition index (DI) results using the Min Mod Millennium computer program. The effects of the diets on these parameters were tested using the two sample T-tests with $P < 0.05$ being statistically significant.

No significant differences were shown in the minimal model parameters between the high and low protein diets. The glucose concentrations of all the horses during both M-FSIGTs fell below their baseline concentrations for a considerable time period. The glucose concentrations of the horses that received the low protein diet were found to drop below baseline earlier ($P = 0.009$) and remain low for longer ($P = 0.043$) than those on the high protein diet. The reason for this difference is unclear. Applying the M-FSIGT and minimal model analysis to this study shows it is an effective way to estimate glucose metabolism and insulin dynamics in the horse. Investigation into hypoglycaemia in horses and its potential effects on the minimal model analysis would be useful in proving the robustness of the use of the minimal model in horses. Development of a recommended small but effective insulin dose for use during the M-FSIGT on insulin sensitive individuals would be useful to help prevent hypoglycaemia and counter regulation mechanisms during testing.

The results of this study did not show any affect of dietary protein on insulin sensitivity but it has allowed for many more questions to be asked about the mechanism by which protein and amino acids could possibly alter insulin sensitivity in horses and other species.

This research was supported by the RIRDC Horse Program

EFFICACY OF ORAL METFORMIN IN INSULIN-RESISTANT PONIES

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With the increasing appreciation of the clinical importance of insulin resistance (IR) in horses and ponies, the goal of horse managers is to prevent or treat IR before the consequences become manifest. While weight management and increased exercise may be effective, there are circumstances where pharmacological intervention may be warranted. Metformin may be a viable treatment for insulin-resistant (I-R) equids because it reportedly enhances insulin action, in humans, without stimulating insulin secretion. The typical I-R equid displays compensated IR where normoglycaemia, or only mild hyperglycaemia, is accompanied by hyperinsulinaemia. Hence the aim to decrease the basal insulin secretion is paramount. Other effects of metformin seen in humans include decreased blood glucose concentrations by inhibiting hepatic glucose production and the intestinal absorption of glucose, weight loss and reduced lipid levels. Adverse effects of metformin in humans are rare.

The primary aim of this study was to determine the effect of oral metformin on insulin and glucose dynamics in I-R ponies using Minimal Model analysis. The study was conducted as a 2-period, 2-treatment cross-over design, with the 2 treatments being metformin (Metforbell 500 mg, CiplaGenpharm Australia, Pty Ltd) (15 mg/kg BW, PO, BID) (M) and control (C). Eight ponies (non-Cushingoid, non-obese [mean BCS 6/9]) were deemed to be I-R based on cresty neck score and the results of a combined glucose-insulin test. The 8 ponies were randomized to the 2 treatment sequences MC and CM with 4 ponies in each treatment sequence. Both periods of the cross-over continued for 21 days, with a frequently-sampled intravenous glucose tolerance test on Day 0 and Day 22 on all ponies. During the study periods, all ponies were individually stabled, with ad libitum access to water and were fed early-cut oaten hay at a rate of 1 to 2% of BW twice daily, rationed so as to prevent any weight fluctuations, and 100 g/day rice bran pellets (CopRice Cool Conditioner, Leeton, NSW) (into which metformin powder was dissolved for the M group). The C animals received the same handling/diet as the M animals, except for a zero metformin dose. The 2 periods were separated by a 21-day wash-out period where the ponies were kept in a communal paddock. Indices to describe insulin sensitivity (SI, the sensitivity of target tissues to insulin-mediated glucose disposal) and glucose effectiveness (SG, the ability of glucose to mediate its own disposal, independently of insulin) were generated after Minimal Model analysis of the plasma glucose and insulin concentrations. Bodyweight, neck circumference and the height of the crest of the neck were also assessed. The data were analysed using Wilcoxon Signed Rank Tests.

After chronic dosing with metformin for 21 days, SG was significantly depressed ($p=0.016$). This suggests a possible negative effect of metformin treatment on GLUT 1 glucose transporters; SI did not change. In contrast, SI was significantly enhanced in the control group ($p=0.039$). Bodyweight, neck circumference and the height of the crest of the neck did not change in either group.

The lack of any clinically beneficial effect of metformin on SI was disappointing but perhaps not unexpected. In a separate study of the pharmacokinetics of metformin in I-R ponies the data suggested that the bioavailability of metformin in horses is poor, and that chronic dosing may not achieve therapeutic blood concentrations. The enhanced SI of the C group also suggests that perhaps restricting feed intake to 2% BW/day may be therapeutic, and that metformin may counteract this effect. This conclusion is guarded because the result was heavily influenced by one member of the group. It is also possible that the mode of action of metformin is associated with BW (adipose tissue) loss and that by having non-obese ponies and titrating our feeding regimen to prevent weight loss, we have inadvertently prevented any effect of metformin on SI.

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THE RELATIONSHIP BETWEEN INSULIN STATUS AND OCD OCCURRENCE IN THOROUGHBRED YEARLINGS

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Insulin resistance is a complex pathophysiological condition that appears to underlie a number of chronic conditions often called 'equine metabolic syndrome', including laminitis obesity, and osteochondritis dissecans (OCD). In the horse, an abundance of literature describes the affects of diet and exercise on insulin resistance, but only a few studies have investigated insulin resistance and bone development. OCD is essentially a developmental disease caused by a defect in the normal process of bone formation resulting in the thickening, cracking and tearing of the joint cartilage of growing horses. OCD is known to be a multi-factorial condition associated with dietary deficiencies and/or nutrient imbalances, biomechanical stress or trauma, rapid growth rates, and genetic influences. In regards to insulin resistance and OCD, there is a current hypothesis that elevated post-feeding blood insulin levels may predispose growing horses to develop OCD (Ralston 1996). However the observed hyperinsulinaemia in these studies may be just reflect excess body weight, rather than a direct cause of OCD.

Recent research suggests interesting links between insulin and bone metabolism. The skeleton is now regarded as an endocrine organ that affects energy metabolism (Reinhr and Roth, 2010), with a key role for the hormone osteocalcin in regulating insulin responses (Fernandez-Real *et al.*, 2009). Other recent findings have shown that the hormone leptin, produced by adipose tissue, is able to regulate bone metabolism via the sympathetic nervous system (Confavreaux *et al.* 2009). Further IGF1, which acts in concert with insulin to promote bone and cartilage growth, also exerts insulin-like affects on carbohydrate metabolism. Therefore perturbations of any of these hormones may contribute to the development OCD in horses.

Our current study investigates the relationship between insulin status and the occurrence of OCD in Thoroughbred yearlings. Briefly, yearlings (52) were recruited from a NSW Thoroughbred stud farm and fasting blood samples were obtained. Plasma insulin and glucose concentrations were determined by standard techniques. OCD and other skeletal abnormalities were determined by radiography. Initial data suggests a relationship between increased insulin sensitivity and the incidence of OCD in these yearlings. Further work is in progress to confirm and extend these findings.

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FOCAL INJURY INDUCES WIDESPREAD PATHOLOGY IN EQUINE SUPERFICIAL FLEXOR TENDONS

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Focal superficial digital flexor tendon (SDFT) injuries are common in performance horses. An incomplete understanding of the pathobiology of tendon degeneration and repair, particularly the non-collagenous matrix, contributes to the limited therapeutic options and poor outcomes. The purpose of this study was to investigate changes in the non-collagenous matrix of the SDFT following focal injury. Lateral hemi-transection of one forelimb SDFT was performed in the mid-metacarpus in six adult (3-19yr) horses. Another three horses had sham surgery in one limb while the contralateral limb was a non-operated-control (NOC). SDFTs were examined by ultrasound pre-operatively and at 2, 4 and 6 weeks post-injury. Horses were housed in small yards until sacrifice 6 weeks after surgery. SDFTs were harvested and divided into 12x3cm regions: 1-4, 4-7, 7-10cm proximal or distal to the medial cut and each of these regions divided into medial (over-stressed) and lateral (under-stressed) tendon halves. Each of the 12 regions was then divided into 3: one each for (i) RNA extraction and biochemistry, (ii) biomechanical testing, and (iii) fixation/histology

By four weeks post-surgery sham-operated horses had no lameness at the walk or trot. Mild lameness at the trot persisted in the partial-transected horses until euthanasia at six weeks post-surgery. Sham-operated and non-operated control tendons showed normal echogenicity and cross-sectional area prior to surgery and at all time-points after surgery. The lesion created by SDFT partial-transection was visible ultrasonographically in the mid-metacarpus at all times. The SDFT 6cm distal to the lesion had normal echogenicity but increased cross-sectional area in all partially-transected tendons. The SDFT 6cm proximal to the lesion had normal echogenicity in three of the partially-transected tendons with the other three showing areas of hypoechogenicity.

Histology: There was no difference between NOC and sham-operated tendons so these were pooled as "controls". The histopathology score was increased throughout cut tendons ($p < 0.03$) except for the most distal regions. The PG score was increased in cut tendons in all areas ($p < 0.02$) except the most proximal under-stressed region. Histopathology but not PG scores differed with distance from the cut (lateral $p = 0.0005$, medial $p = 0.042$), with regions nearest the cut being worst. There was no difference in histopathology or PG scores between stress-deprived and over-stressed tendon halves.

Gene expression:: There was no difference between NOC and sham-operated tendons so these were pooled as "controls". In cut tendons, gene expression changes were grouped into those that: (i) significantly increased - aggrecan, versican, biglycan, collagens-1 & -3, MMP-14 and TIMP-1; (ii) significantly decreased - ADAMTS-4 and MMP-3, or (iii) did not change - decorin, fibromodulin, lumican, COMP, collagen-2, ADAMTS-5 and TIMP-2 & -3. There was a significant difference in the fold change in expression between regions proximo-distal for aggrecan ($p < 0.008$), biglycan ($p < 0.006$) and MMP-3 ($p < 0.007$), with regions nearer the cut showing a greater transection-induced change in expression. There were no differences in expression of any genes between medial/overstressed and lateral understressed tendon halves.

Widespread degenerative changes extending at least 10cm proximal and distal to the SDFT lesion may contribute to poor clinical outcome following focal tendon injury. PG accumulation (aggrecan, versican and/or biglycan) and altered proteinases and inhibitors (decreased ADAMTS-4 and MMP-3 and increased MMP-14 and TIMP-1) may have a detrimental effect on regional tendon biomechanics, and explain the high re-injury rates and poor prognosis for return to previous performance in equine athletes.

A universal theme in tensile tendon degeneration is the development of a fibrocartilaginous phenotype.

MORPHOLOGY OF THE SACROILIAC JOINT IN 37 THOROUGHBRED RACEHORSES

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Degenerative changes have been reported in the sacroiliac joints of Thoroughbred (TB) racehorses and other performance horses with no history of back or pelvic injury. The aim of this study is to document the frequency and type of changes in sacroiliac joint surfaces of TB racehorses with and without a known history of back pain.

Pelvises from 37 TB racehorses that had been declared for euthanasia at the Hong Kong Jockey Club (HKJC) were analysed. Full veterinary medical history including the reason for euthanasia was obtained for each horse. The sacroiliac joint (SIJ) articular surfaces were photographed, measurements taken and the morphology was descriptively analysed. Sacral and iliac joint surfaces were independently graded based on type of joint extensions due to modelling, and degree (mild, moderate, severe) of joint surface adaptations. The surface area of the iliac and sacral joint surfaces was determined from the photographs, using Image J (Image Processing and Analysis in Java). The surface area, shape and type of joint and grade of SIJ changes and symmetry left to right were compared for horses with and without documented presence of back or SIJ/pelvic pain

Differences were observed in SIJ surface adaptive changes, prevalence of severe degree of articular surface changes, type and frequency of joint extensions, and surface features that existed between the subgroup of TBs with a history of back or SIJ pain, and the subgroup of TBs with no back or SIJ pain. There was no significant difference between the two groups for body weight or articular surface area, and no association between age and joint surface features, degree of joint surface adaptive change or SIJ surface area. There was no relationship between body weight and articular surface area. Relationships were found between surface area of joint and degree of joint surface adaptive change ($p < 0.001$), and positive correlation between surface area and degree of joint surface adaptive change ($p < 0.001$).

Differences in the morphology of SIJs regarding SIJ type, shape, symmetry, joint features and degree of joint surface adaptive change were observed between a group of racing TBs with history of back and SIJ pain and a group with no such history.

A COMPARISON OF THE MORPHOLOGY OF THE SACROILIAC JOINT OF AUSTRALIAN BRUMBIES AND THOROUGHBRED RACEHORSES

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The morphology of the sacroiliac joint (SIJ) of Thoroughbred (TB) racehorses has been documented in previous studies. It has been noted that some of the morphological features may be degenerative in nature, or may be related to the training and racing regimen of the TB racehorse. The aim of this study was to compare the morphology of the equine SIJ of the TB racehorse to that of the SIJs of a group of Australian brumbies.

The SIJ articular surfaces of seventeen pelvises from a group of Australian brumbies were photographed, and the morphology described. Measurements were taken from the joint surfaces, and the surface area (SA) of the iliac and sacral joint surfaces was determined from the photographs, using Image J (Image Processing and Analysis in Java). The surface area, shape and type of joint and grade of SIJ changes and symmetry left to right were compared to those noted in a previous study (Goff, 2010) of the morphology of the SIJ of the TB racehorse.

When compared to a population of TBs, there were similar shapes and types of SIJ and joint surface features present in the brumbies. The main differences were in size, surface area, distribution of some of the joint surface adaptive changes, frequency of joint type and degree of joint surface adaptive change. There was no association between age of the horse and joint SA, features, type, or degree of adaptive change.

The results of this study have contributed to the bank of knowledge regarding morphology of SIJ surfaces in horses. There are similarities and differences between SIJs of brumbies that have never been subject to ridden exercise, versus horses that have undergone training and racing.

Goff, L.M. (2010) Morphology of the sacroiliac joint of 37 Thoroughbred racehorses *Proc. Australasian Equine Sc Symp.* 3. In these Proceedings

THE USE OF NON-VETERINARY THERAPIES ON COMPETITION HORSES IN NEW ZEALAND

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The aim of the survey was to obtain baseline data on the use of non-veterinary therapy within competitive equestrian sport in the North Island of New Zealand

Data were collected during January 2010 at the North Island Show jumping Championships, the North Island Dressage Championships and at racing yards in the Central Districts (Manawatu and Wanganui regions) of the North Island. The survey consisted of 30 open and closed questions and was conducted face-to-face, by a single interviewer. Information on the demographics of each discipline (show jumping dressage and racing), the use of non-veterinary therapy and knowledge of training and qualifications of the non-veterinary therapists was obtained. Univariable and multivariable logistic regression was used to identify associations between demographic variables and the use of non-veterinary therapists.

In total, 110/190 riders/trainers participated in the survey, resulting in an overall response rate of 58%. The relative contribution of responses across disciplines was 39/110 (35%), 41/110 (37%) and 30/110 (27%) for show jumping, dressage, and racing, respectively. Non-veterinary therapists were used by 62% of respondents (68/110) to treat their horses. The most common types of non-veterinary therapy used were chiropractic (25/67; 37%) and physiotherapy (16/67; 24%). The main reason for using non-veterinary therapies was for back pain (22/68; 32%). In the final multivariable model, the rider/trainer discipline and the number of horses in training per season were positively associated with the use of non-veterinary therapy.

The data indicates that the use of non-veterinary therapies for the treatment of competition and racehorses is widespread across New Zealand. Many riders/trainers found non-veterinary therapy beneficial, however many therapists and veterinarians do not work together and therefore the holistic treatment approach is lost.

EFFECT OF BANDAGING ON HEALING OF DISTAL LIMB WOUNDS IN HORSESA.J. Dart^A, N.R. Perkins^B, C.M. Dart^A, L.B. Jeffcott^A and P. Canfield^A^A University Veterinary Teaching Hospital, University of Sydney, Camden, NSW, 2570^B AusVet Animal Health Services, Toowoomba Qld, 4350

Despite variable results from different studies, it has been suggested that any form of bandage may lead to production of excessive granulation tissue in horses.¹ Although, the specific cause of excessive granulation tissue is unknown, bandaging is thought to promote the accumulation of exudate, cause persistent inflammation and reduce the ambient oxygen tension which all can stimulate angiogenesis and fibroplasia.^{1,2} However, the effect of bandaging on the healing process in horse limb wounds remains unclear because of differences in study design and objectives. The purpose of this study was to evaluate the effect of a non-occlusive dressing, incorporated in a standard three layer bandage, on second intention healing of distal limb wounds in horses.

Standardised, full thickness wounds were made in the skin overlying the dorsomedial aspect of the metacarpus in 33 horses. In 17 horses wounds were bandaged with a non-occlusive dressing covered by gauze coated cotton wool and adhesive bandage until wounds healed. In 16 horses wounds were left unbandaged to heal. Wounds were digitally photographed 24-48 hours after wound creation, then weekly for 9 weeks. Images were analyzed using image analysis software. Comparisons of wound size (cm²), total days to healing, and rate of wound healing (cm²/day) were made between bandaged and unbandaged wounds.

There were significant effects associated with bandage ($P < 0.0001$), week ($P < 0.001$), and bandage by week interaction ($P < 0.0001$). There was no difference in wound area at the first recording time point after wound creation ($P = 0.38$). After week 1, there was a difference at each time point out to week 9 when weekly recording ceased. Bandaged wounds had greater and more prolonged retraction after wound creation. Open wounds retracted for 2 weeks before starting to undergo contraction while bandaged wounds continued to retract for 3 weeks. No unbandaged wounds required excision of excess granulation tissue while bandaged wounds required regular trimming. There was no difference in the total days to healing ($P = 0.14$) or overall healing rate ($P = 0.43$) between bandaged and unbandaged wounds.

Bandaging of distal limb wounds in the horse with a non-occlusive dressing incorporated in a standard 3 layer bandage commonly used in horses alters the pattern of healing and promotes the production of excessive granulation tissue. However if the excessive granulation tissue is regularly excised, there is no effect on ultimate time to healing.

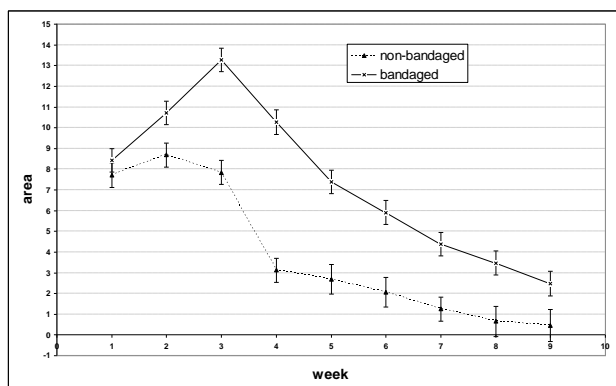


Figure 1. Least square means for the bandage x week interaction term. The horizontal axis shows weeks (1 through 9) and the vertical axis shows wound area (cm²) with different lines showing means for the bandaged and non-bandaged groups. Bars represent one standard error (sem) either side of the mean.

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THE EFFECT OF MANUKA HONEY ON SECOND INTENTION HEALING OF CONTAMINATED AND UNCONTAMINATED WOUNDS ON EQUINE DISTAL LIMBS

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Wounds on the distal limb of horses are predisposed to exuberant granulation tissue formation and prolonged healing. A variety of topical agents aimed at enhancing wound healing in horses have been evaluated. Manuka honey has broad spectrum antibacterial activity and has been reported to stimulate an inflammatory response in leucocytes contributing to a beneficial effect on wound retraction and contraction. In a preliminary study, manuka honey has been shown to modulate healing of equine distal limb wounds. The aim of this study was to evaluate the effect of daily application of manuka honey for 12 days (short term) or until healed (long term) on wound area and overall healing rate of contaminated and non-contaminated distal limb wounds in the horse. To apply manuka honey long term without a bandage a water based manuka honey gel containing 66% honey was formulated.

In 10 horses, 5 full thickness skin wounds (2cmx2cm) were created on the third metacarpus of both front limbs. Limbs were randomly assigned to contaminated or non-contaminated groups. To create contaminated wounds, horse faeces were applied to the wounds for 24 hours. All wounds were bandaged. After 24 hours wounds were randomly assigned to 5 different treatment groups; untreated (control) wounds; manuka honey, honey gel (short and long term), and gel alone. For 12 days bandages were changed daily and wounds were treated. On day 13 bandages were removed and wounds were left open to heal. On one wound (long term), treatment with the honey gel mixture was continued until the wounds were healed. The wound area was measured on day 1, 7, 14, 21, 28, 35 and 42. Overall time to healing was recorded for all wounds. The wound area on each recording day and rate of healing was compared statistically between treatment groups and between contaminated and non-contaminated limbs.

The mean wound area (cm²) of all wounds was not different on day 1. Wounds were treated with honey or honey gel were smaller on days 7, 14, 21, 28 and 35 compared with untreated and gel treated wounds. From day 7 to 28, the mean wound healing rate (cm²/day) between wounds treated with honey or honey gel and the untreated and gel treated wounds was not different, however contaminated wounds healed faster than non-contaminated wounds. From day 28 to 42 the wound healing rate was faster in honey and honey gel treated wounds compared to untreated and gel treated wounds but there was no difference in the rate of healing between contaminated and non-contaminated wounds. Overall wound healing rate was faster for honey and honey gel treated wounds compared to the untreated and gel treated wounds. Long term application of honey gel improved the overall rate of healing compared to all other treatment groups. The overall healing rate for contaminated wounds was faster than for non-contaminated wounds.

Manuka honey and manuka honey gel improves healing of distal limb wounds left to heal by second intention in horses.

SERUM IRON CONCENTRATIONS AS INDICATORS OF SEPSIS AND SIRS IN CRITICALLY ILL NEONATAL FOALS

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Septicaemia is a leading cause of death or admission to a Neonatal Intensive Care Unit (NICU) in equine neonates. The early signs can be subtle and the neonatal foal does not always react with predictable changes in clinical or laboratory parameters. The gold standard to diagnose septicaemia in the neonate is a microbial isolate grown from blood culture. Blood culture results are not readily available and less than 25% yield a microbial isolate. Due to the potentially rapid deterioration of the untreated septicaemic foal other laboratory measurements are required to aid diagnosis. Serum iron concentrations ([Fe]) have been shown in adult horses to decrease with acute onset of Systemic Inflammatory Response Syndrome (SIRS) and following surgical trauma. This reduction can be seen prior to the change of inflammatory indicators such as white blood cell count (WBCC) and fibrinogen measurement.

Blood samples were obtained from 10 normal thoroughbred foals (Healthy) at 1 hr, 12 hrs and 24 hrs of age, and analysed for serum [Fe]. The records from 241 foals (NICU) admitted to Scone Equine Hospital between September 2008 and December 2009 were reviewed. The following data was recorded: signalment, admission physical examination and clinicopathological data. Sepsis score was calculated. Sepsis was defined as foals with either a positive blood culture, a sepsis score of ≥ 14 or microbiological evidence of sepsis. SIRS was defined as \geq two of the following; abnormal temperature ($<37^{\circ}\text{C}$ or $>38.5^{\circ}\text{C}$), abnormal WBCC ($<5.2 \times 10^9/\text{L}$ or $>12 \times 10^9/\text{L}$) or presence of band neutrophils. JMP 7 (SAS Institute Inc, Cary NC, USA) was used for statistical analysis and the ANOVA and Tukey's test, Wilcoxin Rank Sum, Contingency analysis and Fishers Exact Test, or Logistic Regression was used as appropriate.

The mean serum [Fe] of healthy foals decreased linearly between 1hr ($389.6 \mu\text{g/dL} \pm 47.1$), 12 hrs ($302.7 \mu\text{g/dL} \pm 36$) and 24 hrs ($222.2 \mu\text{g/dL} \pm 50.7$) of age ($p=0.001$). The NICU foals serum [Fe] also decreased with age and accordingly were grouped as follows; 6 hrs or less (66 foals), >6 to 18 hrs (53 foals), >18 to 36 hrs (30 foals) and greater than 36 hrs (92 foals). The serum [Fe] was different in all NICU foal age groups. Logistic regression showed that serum [Fe] was a good parameter to assess foal health immediately after birth. NICU foals <6 hrs were more likely to have a lower [Fe] than Healthy foals at 1 hr of age ($p=0.004$, Odds Ratio 0.99) with the area under the receiver operator characteristic curve of 0.75 and an optimal sensitivity and specificity of 329 $\mu\text{g/dL}$. The serum [Fe] of the NICU groups (>6 to 18 hrs, >18 to 36 hrs) and age matched Healthy foals (12 and 24 hrs) was similar. NICU foals <6 hrs with Sepsis had a lower [Fe] (263 $\mu\text{g/dL}$, Inter Quartile Range (IQR) 163 -354 $\mu\text{g/dL}$) than foals that did not have Sepsis (336 $\mu\text{g/dL}$, IQR 300-381 $\mu\text{g/dL}$) ($p=0.009$). NICU foals <6 hrs with SIRS had a lower serum [Fe] (268 $\mu\text{g/dL}$ IQR 192-318 $\mu\text{g/dL}$) than foals that did not have SIRS (331 $\mu\text{g/dL}$, IQR 278-395 $\mu\text{g/dL}$) ($p=0.026$). Foals >36 hrs with SIRS had a lower serum [Fe] (46 $\mu\text{g/dL}$ IQR 20-78 $\mu\text{g/dL}$) than similarly aged foals without SIRS (81 $\mu\text{g/dL}$ IQR 35-142 $\mu\text{g/dL}$) ($p=0.0366$). The positive predictive value of a serum [Fe] less than 329 $\mu\text{g/dL}$ for Sepsis and SIRS was .57 and .42 respectively but the negative predictive value was .74 and .85 respectively.

Serum iron concentrations decrease rapidly in the first 24 hours in the newborn healthy foal. Serum iron concentrations less than 329 $\mu\text{g/dL}$ can be used in the neonatal foal less than 6 hours old to assist in the early diagnosis of SIRS and Sepsis thus allowing prompt instigation of appropriate therapy in approximately 57% of foals. Low serum iron concentrations can also be used to aid the diagnosis of SIRS in foals older than 36 hours.

CAUSES OF FOAL PERINATAL MORTALITY IN NEW ZEALAND, 2007-2009*E.K.Gee^A, A. Wolfe^A, A.C. Johnstone^A, I.G.Mayhew^A and E.C.Firth^A*^A Institute of Veterinary, Animal and Biomedical Sciences, Massey University, Palmerston North, New Zealand

Late-term abortions and perinatal deaths result in major losses to the equine industry; however, there are few studies reporting the causes of such losses in New Zealand. Most published work on late-term abortions and perinatal death originates in the North Hemisphere. In the United Kingdom the majority of losses are attributed to umbilical cord lesions, dystocia, placentitis and unknown causes (Smith et al, 2003), while recent data from the United States attributed most losses to infectious agents, musculoskeletal problems, and gastrointestinal problems (Sturgill & Carter, 2009). The objective of our study was to identify the major causes of late abortions and perinatal losses in New Zealand.

Data were collected from pathological investigations on late-term fetuses and foals that died within the first month of life. Foals were collected from the Manawatu and Waikato regions during the 2007 and 2008 foaling seasons and from the Manawatu region during the 2009 foaling season. Foals were classified as stillborn (born dead in the last month of pregnancy), neonatal (born alive but died within 2 days of birth) or post-natal (more than 2 days old at time of death).

In total 76 foals were received: 67 Thoroughbred, 3 Warmblood, 2 Clydesdale, 3 Miniature horses and 1 Welsh pony. The major causes of death are summarised in Table 1.

Table 1 Summary of causes of death from foals necropsied during the 2007 and 2008 foaling seasons (n=59) and 2009 foaling season (n=17).

Season and Status	Infectious	Dystocia	Congenital	Other	Unknown	Total
2007&2008						
Stillborn	4	9	1	1	3	18
Neonate	3	4	3	2	5	17
Post-natal	20	0	0	3	1	24
2009						
Stillborn	0	7	1	3	1	5
Neonate	0	0	1	1	1	10
Post-natal	2	0	0	0	0	2
Total	29	20	6	10	11	76

Deaths from infectious agents comprised the largest group of foals, with the majority of these cases (n=26) occurring in the post-neonatal group. Nine cases were due to enterocolitis, colitis or peritonitis, 7 had pneumonia, and 6 had septicaemia affecting multiple organs. Etiologies included Streptococcal placentitis (n=2), equine herpes virus-1 (n=3) and Actinobacillus equuli septicaemia (n=1). Twenty foals died due to dystocia: in some cases this diagnosis was made on the basis of history only as histopathological lesions that result from intraparturient anoxia and death can be very subtle (if present). Nine cases classified as dystocia had cardiac bruising. The cause of death for 11 cases was undiagnosed, often due to advanced autolysis (n=6) or poor history.

The results of our study are similar to other studies conducted in the Northern Hemisphere with different production systems and foaling mare management, wherein 38% of foal deaths have been associated with the presence of infectious agents. The high proportion of deaths associated with dystocia (26% of foals in this study) warrants further investigation.

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AGE INCIDENCE OF DIARRHOEA IN FOALS

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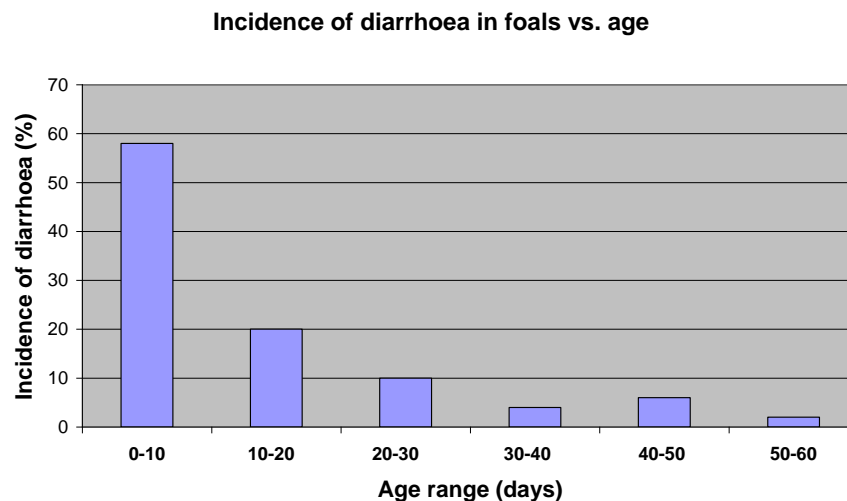
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Diarrhoea in the equine neonate is an ongoing problem which can be a significant cost to industry. The aim of this study was to determine the age incidence of diarrhea in foals less than 60 days old and to identify “at risk” periods which may ultimately be associated with distinct aetiologies.. It will be seen that death due to foal diarrhoea is extremely rare where management is of a very high standard.

The clinical records for the first 60 days of life of 1648 foals, born during the a seven year study period, were reviewed. All foals had been inspected at least twice daily and any abnormalities were recorded. Any foal observed as having a wet tail or seen passing fluid faeces on any occasion was recorded as having diarrhoea. All deaths, variables and treatments were recorded. Some cases were transient returning to normal within a few hours without any treatment while others continued for variable periods of time.

Diarrhoea was recorded in 478 cases , representing 29% of the foals born but very few cases (< 10%) were shown, by laboratory analyses, to be caused by known bacterial or viral pathogens. The low number of confirmed diagnoses is an indication of how difficult it has been to confirm the aetiology of foal diarrhoeas. Allocating the foals into ten day groups (i.e. 1-10 days of age, 11-20 days of age, etc.) it can be seen that there is a significant age distribution (>0.005). This can also be illustrated graphically.



Of the foals with diarrhoea 35.3% were in the neonatal period (birth to 5 days), 27.8% around the foal heat period (6-12 days), a further 20.3% in the period from 13-26 days and the remaining 16.6% being in older foals from 27-60 days. Analysis of the background history showed a correlation was observed between use of a veterinary antibiotic and the onset of scouring.

The incidence remained remarkably constant with an overall percentage of 29% of foals developing diarrhoea in each year of the 7 year study. There was a constant increase in the population during this time with a steady annual increase in foal numbers. Interestingly this is consistent with results from Californian foals over a similar period (D.Wilson Personal communication).

A complementary study was conducted to investigate possible causes of foal diarrhoea, in which paired healthy and scouring foals were sampled (a total of 21 foals; 9 healthy and 12 scouring). Samples were analysed for microbial diversity and Lactobacilli in particular. Overall these samples showed:

- A preponderance of *E. coli*, raises the potential implication of pathogenic *E. coli* as a cause of disease.
- The presence of an as-yet unidentified class of organisms in 7 of the 12 scouring foals, which may be a causative agent or a marker organism for disease.
- Absence of Lactobacilli (an indicator of intestinal health) from most of the samples, indicates that supplementation with a registered probiotic product such as Protexin[®] would be warranted in newborn foals.
- Presence of *Clostridium difficile* in six foals, highlights the need for good hygiene on the part of stud staff, especially those receiving antibiotic therapy.

BREATH SAMPLING IN NEONATAL FOALS FOR PREDICTION OF *RHODOCOCCLUS EQUI* INFECTION

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Rhodococcus equi infection in foals is a commonly recognised problem in the horse breeding industry worldwide. It continues to pose major challenges with respect to epidemiological and therapeutic control of disease since no effectively protective, safe vaccine is available for use in foals. This project investigated the use of exhaled breath samples from neonatal foals as a means of determining whether exposure to virulent *R. equi* in the first 10 days of life had (i) any relationship to subsequent *R. equi* infection and disease development in foals, or (ii) an influence on the environmental burden of virulent *R. equi*.

By using a portable air sampling device held in the respiratory zone of foals, exhaled breath was collected onto selective agar plates for microbiological culture and subsequent DNA hybridisation to differentiate between virulent and avirulent strains of *R. equi*. Background air samples were collected to compare the concentration of virulent *R. equi* exhaled by foals to that in the surrounding environmental air.

The exhalation of virulent *R. equi* was detected in some but not all neonatal foals. There was no significant relationship between the detection of exhaled virulent *R. equi* from breath samples of neonatal foals and the subsequent diagnosis of rhodococcal pneumonia in individuals. The median concentration of virulent *R. equi* in the exhaled breath of neonates was not significantly different from that of the surrounding air over the study period. Virulent *R. equi* was detected in exhaled breath samples from some, but not all, older foals (1-2 months old) and was found to have no significant relationship to the expression of disease as determined by ultrasonographic examination of the thorax and clinical signs in individual foals. The median concentration of virulent *R. equi* in exhaled breath of 1-2 month old foals was not significantly different from that of the surrounding air over the study period. The proportion of 1-2 month old foals exhaling virulent *R. equi* was, however, significantly greater than the proportion of neonatal foals exhaling virulent *R. equi*.

The results of this study show that the use of exhaled breath samples by a portable air sampling device for the prediction of rhodococcal pneumonia is of no value in individual animals on farms where early screening techniques for disease detection are already employed. Using the portable air sampling device for breath sampling has previously been shown to be of value in herd situations and in environmental studies for detection of virulent *R. equi* in air, however it has no value as a positive predictor of disease in individuals.

DO FOALS CONTRIBUTE TO THE TRANSMISSION OF VIRULENT *RHODOCOCCLUS EQUI*?

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Aims

This study investigated the level of aerosolised *Rhodococcus equi* in holding yards before, during and after mustering of mares and foals.

Methods

Air samples were collected in the yards used to hold mares and foals waiting for veterinary or other husbandry procedures. Selective bacterial culture, colony blotting and DNA hybridisation methods were used to quantitate the levels of aerosolised virulent and avirulent *R. equi*. Air samples were collected between October 2004 and March 2005 from 4 farms that had previously reported endemic rhodococcal pneumonia in foals. Air samples were collected from the yards prior to commencement of mustering, while the mares and foals were running into the yards and then again after the mares and foals had been held in the yards and the dust had settled. Samples from within the mob were collected from the respiratory zone of foals within the mob.

Results

The median level of both virulent and total *R. equi* was significantly greater in samples collected from within the mare and foal mob than from either the samples collected from the yards at rest prior to mustering, or from samples air collected during mustering.

Conclusions

Foals are exposed to greater levels of both virulent and avirulent *R. equi* while they are confined in the yards than they are during mustering. While the concentration of aerosolised *R. equi* is an important determinant of development of rhodococcal disease, these data suggest that the foals in the mob also may be a significant source of exposure, in addition to the virulent *R. equi* burden generated from the soil environment.

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INVESTIGATING SEQUENCE CHANGES IN EQUINE HERPESVIRUS 1 NEUROLOGICAL DISEASE

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Equine herpesvirus 1 (EHV-1) is an alphaherpesvirus that is a major cause of respiratory disease and late term abortion in horses. Less frequently, EHV-1 causes a serious neurological disease. EHV-1 neurological disease has been reported with increased frequency in the USA and Europe in recent years. In contrast, the prevalence of EHV-1 neurological disease in Australia has remained consistently low.

The neuropathogenic potential of EHV-1 has been shown to be strongly associated with a single nucleotide change in ORF30, which leads to an amino acid change in the highly conserved catalytic subunit of the viral DNA polymerase. To investigate the frequency of EHV-1 strains encoding the neuropathogenic DNA polymerase, the catalytic subunit of the DNA polymerase gene of 58 Australian EHV-1 isolates was sequenced. These viruses were isolated from cases of abortion (55 isolates) and neurological disease (3 isolates) between 1977 and the present day. Two of these 58 viruses contained the neuropathogenic DNA polymerase sequence: one of these was isolated from aborted foetal tissue and the other from a case of neurological disease.

Recent data suggests that other changes in the EHV1 genome may also affect the neuropathogenic potential. To further understand our Australian isolates the entire ORF30 gene was sequenced and a number of novel amino acid changes were found.

These results are consistent with other studies showing the association of the single nucleotide polymorphism in abortigenic isolates of EHV-1. The prevalence of neuropathogenic isolates found in abortion outbreaks is similar to that seen in other studies carried out in the northern hemisphere. The prevalence of neuropathogenic isolates in the general Australian horse population is currently being investigated.

Determining the frequency of isolates with neuropathogenic potential in archived Australian EHV-1 isolates will enable future comparisons with EHV-1 current Australian isolates, as well as with isolates where the prevalence of neuropathogenic EHV-1 disease has been recently increasing.

PATHOGENESIS OF AN APHTHOVIRUS INFECTION OF HORSES; PERSISTENT URINARY SHEDDING FOLLOWING EQUINE RHINITIS A VIRUS INFECTION

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Equine rhinitis A virus (ERAV) is a Picornavirus, classified with foot-and-mouth disease virus in the *Aphthovirus* genus. The viral genome is single stranded, positive sense RNA. ERAV infection causes an acute, febrile respiratory disease in horses, with sub clinical cases also observed. The significance of ERAV as a pathogen of horses has likely been underestimated due to difficulties in isolating this virus in cell culture, and many details regarding the pathogenesis remain unknown.

To investigate the pathogenesis of ERAV infection, two horses were experimentally infected with one of two ERAV isolates. Clinical samples including blood, urine and nasopharyngeal swabs were collected for up to 37 days post infection. Despite finding significant quantities of virus in nasal secretions and blood, clinical signs associated with an acute febrile respiratory disease were not observed in either horse during the trial. A surprising finding was however, the prolonged shedding of ERAV at high titres in the urine post infection. To investigate this further, the prevalence of ERAV in post-race horse urine samples was investigated using a quantitative reverse transcription PCR assay.

Table 1 Equine rhinitis A virus prevalence in post-race horse urine samples collected by Racing Analytical Laboratories, Flemington, Oct-Nov 2009

	Age (years)			Total
	2-4	5-6	>7	
No. samples	143	56	16	215
Prevalence (n)	29% (42)	10% (6)	12% (2)	23% (50)

ERAV was most prevalent in urine samples collected from horses aged between 2 - 4 yrs (29%, 42 out of 143). A high level of virus was detected in 44% of positive samples with cycle threshold value of less than 25. Virus shedding was detected in all sexes and infectious virus was isolated.

The nucleic acid sequence of a variable region within the genome for a proportion of virus isolates was determined. Phylogenic analysis of these sequences suggests variants of a single virus isolate are circulating within this horse population.

Currently the laboratory is investigating any clinical implications associated with chronic virus shedding and the mechanisms of viral persistence.

DUAL INFECTIONS AND REASSESSMENT OF SEROTYPE CLASSIFICATION IN ERBOVIRUSES

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Erboviruses are picornaviruses associated with respiratory disease in horses. Equine rhinitis B virus (ERBV) is the sole species. Three serotypes have been identified: ERBV1, ERBV2 and ERBV3. Phylogenetic analysis based on the amino acid sequence of the capsid proteins (P1 region of the genome) assign erboviruses to three distinct phylogenetic groups that correspond with acid stability phenotype but have not, until this time, correlated with serotype. Both acid-labile and acid-stable phenotypes of ERBV1 are recognised, while ERBV2 are acid-labile and ERBV3 are acid-stable. The genomic sequences of the acid-stable ERBV1s closely resemble that of ERBV3. In this study, ERBV1, ERBV2 and ERBV3 specific antisera was used to examine the relationship between serotype, genotype and acid stability phenotype. The occurrence of dual serotype infections was also investigated.

Hyperimmune rat sera against acid-labile ERBV1, acid-stable ERBV1, ERBV2 and ERBV3 were prepared and used in virus neutralisation assays with ERBV isolates. The rat sera were used to test isolates to confirm serotype, particularly of the virus considered to be 'acid-stable ERBV1'. Cross-neutralisation between acid-stable ERBV1 and ERBV3 indicated these viruses were the same serotype. Analysis of three other available ERBV isolates considered to be acid-stable type 1 showed strong cross neutralisation antibody titres with the ERBV3 and acid-stable ERBV1 antisera and little to no neutralisation by the acid-labile ERBV1 antisera. These results show that the viruses previously classified as acid-stable ERBV1 are in fact ERBV3. Erboviruses now clearly segregate into three distinct groups consistent with genotype, serotype, and phenotype - acid-labile ERBV1, acid-labile ERBV2 and acid-stable ERBV3.

Viruses were treated at pH 3 or with ERBV3 antiserum to reveal dual infection with acid-stable and acid-labile viruses. Dual infections were identified in three samples. All were found to contain both ERBV1 and ERBV3. This is the first reported occurrence of an infection with multiple ERBV serotypes in a single horse. Additionally, this is the first reported isolation of ERBV3 in Australia. These results suggest dual infection may be common and may account for the previously documented cross-neutralisation of these viruses, particularly with ERBV1 and ERBV3.

PRELIMINARY PHARMACOKINETICS OF ACEPROMAZINE AND ITS METABOLITE HYDROXYETHYLEPROMAZINE SULFOXIDE

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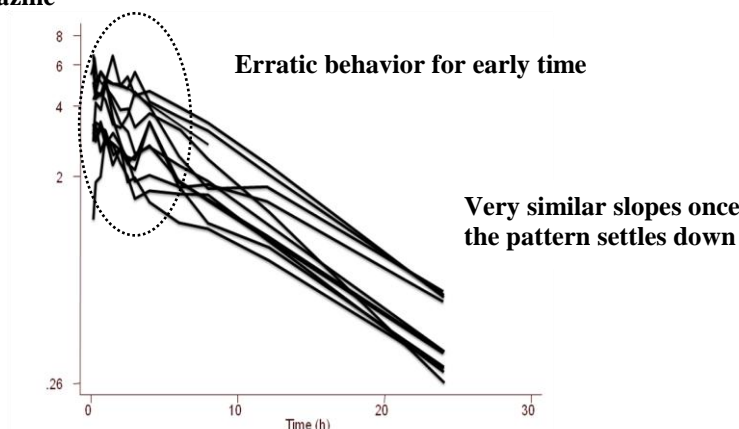
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Acepromazine is prohibited in competition by racing commissions and horse competition organisations, due to its tranquilizing effects. Most pharmacokinetics of APZ has concentrated on the parent drug. However, upon administration, APZ is rapidly converted to hydroxyethylpromazine sulfoxide (HEPS). This study reports both the pharmacokinetics of acepromazine and the metabolite HEPS. The half-life of HEPS is considerably longer than APZ in both blood and urine, making it a more reliable indicator that a horse has been administered APZ within 48 hours of competition.

Twelve geldings of thoroughbred (4) or standardbred (8) breeding were administered a single I.V. injection of 3 ml (30 mg) acepromazine (A.C.P. 10; Delvet Pty Ltd). Baseline blood and urine samples were taken just prior to administration. Blood samples were then taken at 5, 10, 20, 40, 60, 90, 120, 150 min and 3, 4, 6, 8, 12, 24, 32, 48, 72, 96, 120 and 144 h post-administration. These were centrifuged at 4°C and 3,500 x g for 10 min and plasma harvested. Urine samples, collected by free void, were taken at 2, 4, 6, 8, 12, 24, 32, 48, 72, 96, 120 and 144 h post-administration. All samples were stored at -20°C until assayed. For the first 48 hours, horses were fitted with equine nappies in order to collect the total volume of urine. Samples were analysed using LC/MS (Applied Biosystems API 4000 Q-trap, Mulgrave, VIC.). Pharmacokinetic modelling was carried out using WinSAAM software (University of Pennsylvania, PA, USA).

Figure 1. Early chaotic appearance of plasma HEPS (µg/L) in horses (n=12) given an I.V. injection of 30 mg acepromazine



Previous work suggests APZ in plasma and urine is present for up to 120 hours. However, in this study, plasma APZ was completely metabolised after 3 hours in some horses with little APZ reaching the urine unmodified. The appearance of HEPS is quite chaotic in the first hour post-administration (Figure 1) and may be linked to the neurological nature of the primary effect of APZ. Some basic representative pharmacokinetic parameters are summarised in Table 1.

Table 1. Basic representative pharmacokinetic variables for APZ and HEPS in 12 horses. * Values are the same.

Metabolite	α (L/h)	β (L/h)	γ (L/h)	$t_{1/2\alpha}$ (h)	$t_{1/2\beta}$ (h)	$t_{1/2\gamma}$ (h)	Vd (L)	Cl (L/h)
Plasma APZ	13.04	0.314	n/a	0.053	2.21	n/a	468	3130
Plasma HEPS	*	*	0.127	*	*	5.58	633	80

The quantity of APZ in urine was negligible; urinary APZ C_{\max} did not exceed 6 ng/mL compared with urinary HEPS C_{\max} reaching as high as 1500 ng/mL. The detection of HEPS in urine extended to 144 h post administration.

This research was supported by Rural Industries Research & Development Corporation (RIRDC).

THE METABOLISM OF SOLU-CORTEF®: SUBSEQUENT DISTRIBUTION OF HYDROCORTISONE HEMISUCCINATE AND HYDROCORTISONE

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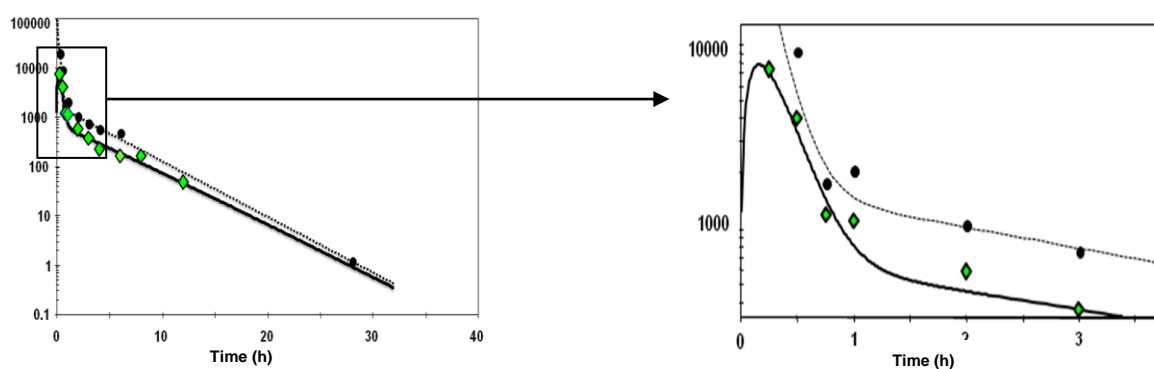
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Naturally occurring glucocorticoids (hydrocortisone (HC) and cortisone), and their synthetic analogs are primarily used for their potent anti-inflammatory effects. Hydrocortisone sodium succinate (HCSS), the water-soluble sodium succinate ester, has similar metabolic and anti-inflammatory actions as HC. Under the Rules of Racing, and in other equestrian competitions, glucocorticoids are classed as prohibited substances. Hence the necessity in being able to reliably detect their presence in the competitive horse.

Upon i.v. administration of HCSS, hydrocortisone-21-hemisuccinate (HSHC) (the free acid of the pharmaceutical dosage form), is detectable in relatively high concentrations before it is finally hydrolysed to HC. The metabolism of HSHC is explored here.

In this study, 1g HCSS, (Solu-Cortef® Pfizer, West Ryde, Australia) was administered i.v. to 10 horses of standardbred or thoroughbred breeding, bodyweight 545 ± 14 kg. On the administration day, blood samples were taken at $t = 0, 15, 30, 45, 60$ mins and 2, 3, 4, 6, 8, and 12 h. These were centrifuged at 4°C and $3,500 \times g$ for 10 min and plasma harvested. Sampling continued twice daily for the next 6 days. Samples were stored at -20°C until assayed. Analysis for HCHS and HC was carried out by LCMSMS. Pharmacokinetic modelling was carried out using WinSAAM software (University of Pennsylvania, PA, USA). A typical model is shown in Figure 1; notice the close convergence of HCHS with HC.

Figure 1. Modelling of HCHS (dotted line) & HC (solid line). Concentrations are $\mu\text{g/L}$.



Some preliminary representative pharmacokinetic variables are presented in Table 1. Values for $t_{1/2}$ are determined by $\log 2/\text{macro rate constant}$, respectively, e.g. $t_{1/2\alpha} = \log 2/\alpha$.

Table 1. Preliminary representative pharmacokinetic variables for HC and HCHS in 10 horses.* Values are the same

Metabolite	α (L/h)	β (L/h)	γ (L/h)	δ (L/h)	$t_{1/2\alpha}$ (h)	$t_{1/2\beta}$ (h)	$t_{1/2\gamma}$ (h)	$t_{1/2\delta}$ (h)	Vd (L)	Cl (L/h)
Plasma HCHS	6.38	0.259	n/a	-	0.1087	2.68	n/a	n/a	10.088	45.2
Plasma HC	*	*	6.57	0.258	*	*	0.1055	2.43	10.055	51.2

The kinetics of HC are influenced not only by the administration of HCSS but also by endogenous production. Surprisingly, endogenous HC secretion was not suppressed by the synthetic glucocorticoid. Confident modelling of the data could only be done using sampling data up to 8 h before the influence of endogenous HC became too great.

This research was supported by Rural Industries Research & Development Corporation (RIRDC)

EVALUATION OF COMMERCIALY-AVAILABLE CORTISOL ASSAYS BEING USED TO MEASURE EQUINE CORTISOL

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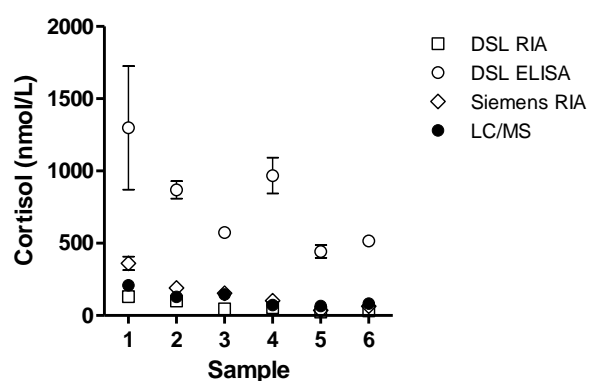
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Equine Cushing's syndrome (ECS) or hyperadrenocorticism is frequently diagnosed in aged horses and ponies. It is due to a primary pituitary pars intermedia adenoma, resulting in excessive secretion of ACTH. Prolonged cortisol excess can lead to insulin resistance (IR), which has been found in horses affected with ECS. Insulin resistance in the horse, in turn, may be associated with laminitis; and chronic insidious-onset laminitis is a predominant clinical complication of ECS. In classic cases, basal serum cortisol levels are often very high. However, in some cases dynamic tests of pituitary function are needed, such as the overnight dexamethasone suppression test (DST), to confirm the diagnosis. Normal horses show significant suppression of serum cortisol concentrations following administration of dexamethasone, unlike ECS cases. Therefore, the accurate measurement of plasma cortisol concentrations in equids is important. Most commercial kits used are designed for human clinical medicine, but there is good reason to anticipate cross-reactivity based on the highly conserved nature of cortisol among mammalian species.

Three commercially-available cortisol assays were evaluated using 6 equine lithium-heparin plasma samples, collected from previous studies, held in our laboratory. The handling/storage of the samples was carefully controlled to allow robust comparisons between the assays. The Siemens Coat-A-Count Cortisol Radioimmunoassay (RIA) (Siemens Medical Solutions Diagnostics, Los Angeles, CA) is widely used to quantify cortisol in equine plasma, as is the DSL-2100 Cortisol RIA (Diagnostic Systems Laboratories, Inc. Webster, Texas). The DSL 10-2000 Cortisol Enzyme-Linked Immunosorbent Assay (ELISA) is new to the market. The cortisol concentrations of the samples were also determined using Liquid Chromatography/Mass Spectrometry (LC/MS), providing a 'gold standard' of measurement.

Data were analysed using Lin's Concordance Correlation Coefficient. Compared to the LC/MS measurement of the cortisol concentration of each sample, the Siemens RIA rendered a good index of correlation ($\rho_c = 0.681$) and the DSL RIA rendered a fair index of correlation ($\rho_c = 0.470$). The index of correlation rendered by the DSL ELISA was poor ($\rho_c = 0.038$) (Figure 1).

Figure 1. Cortisol measurements (Mean \pm SD of duplicates) of 6 equine lithium-heparin plasma samples, after analysis with the Siemens RIA, the DSL RIA, the DSL ELISA and LC/MS.



As the diagnosis of ECS may severely impact the subsequent management of the horse/pony, the accurate measurement of plasma cortisol concentrations in equids is important. It is clear that the Siemens RIA was adequately comparable to GC/MS and is a reliable alternative if GC/MS technology is not available.

This research was supported by the WALTHAM Centre for Pet Nutrition and RIRDC

RECOMBINANT EQUINE GROWTH HORMONE (eGH): A THERAPY FOR OBESITY?

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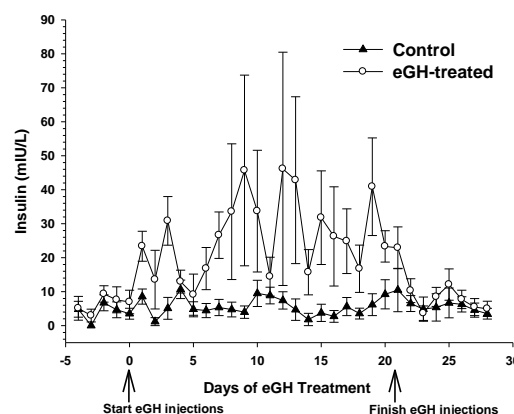
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Obese ponies are often insulin resistant, display hyperinsulinaemia, and are more prone to laminitis than lean animals. Insulin stimulates glucose uptake and metabolism in adipose tissue, leading to lipogenesis, which favours obesity. Conversely, growth hormone (GH) is a catabolic hormone in adipose tissue. By antagonising the action of insulin, GH promotes lipolysis, releasing free fatty acids which are then used to supply energy. Further, GH stimulates the production of insulin-like growth factor-1 (IGF-1), which mimics the effect of insulin, to some extent, by stimulating glucose uptake. This should, in theory, reduce the need for insulin secretion. In practice, however, GH has been shown to induce insulin resistance (IR) in some species after prolonged use. Thus, while GH may have potential for use in obese horses to aid in fat loss, it is important to know the risk of inducing IR, exacerbating hyperinsulinaemia and thus increasing the likelihood of laminitis.

Twelve unfit geldings, aged 2 to 13 yr (6.3 ± 3.4 yr, BW 460 ± 15.8 kg) underwent a training regimen on a high-speed treadmill. They were worked 6 d/wk for 9 wk at heart rates of 150 to 160 bpm, for 10 min (initially) to 30 min by Week 9. In Week 4, the horses were randomly divided into 2 groups. Group A received daily injections of sterile water (2 to 5 mL i.m.); Group B received daily injections of eGH (Bresagen, Adelaide, SA). A dose of 5 mg/d was given for the first 5 d, followed by 12.5 mg/d for a further 16 d. Blood samples were taken daily by venipuncture at 0700 h before feeding or exercise for the duration of the study. Blood was collected into plain glass tubes, allowed to clot, then centrifuged at 4°C and 3,500 x g for 20 min. Serum was harvested and stored at -20°C. Samples were assayed for insulin concentration using the Siemens Count-a-Coat radioimmunoassay. Data were analysed using the *t*-test for unequal variances (Welch's correction).

Figure 1. Mean serum insulin concentrations (\pm SE) of control horses (Group A) and horses treated with daily injections of eGH (Group B).



There was no difference in serum insulin between the 2 groups prior to horses being administered eGH (Days -4 to 0); Group A: 4.0 ± 1.1 mIU/L vs Group B: 6.4 ± 1.1 mIU/L, $p = 0.16$. During the eGH treatment, there was a significant increase in serum insulin ($p < 0.0001$) in Group B, reaching a maximum of 46.2 ± 34.3 mIU/L by Day 12, as shown in Figure 1. Consequently, under the influence of exogenous GH, horses showed significant hyperinsulinaemia, although individual responses to eGH injections were varied. Once eGH administration ceased, serum insulin fell rapidly to concentrations comparable to the control group.

Although the administration of eGH did lead to elevated IGF-1 concentrations, there was no effect in reducing serum insulin concentrations. Instead, insulin concentrations were likely influenced directly by eGH injections, leading to an increase in serum insulin. This effect would be detrimental to equids already presenting with hyperinsulinaemia, suggesting that eGH therapy in such horses or ponies is contraindicated. While long-term administration of eGH may ultimately lead to a reduction in serum insulin, due to changes in body composition, the risk of such animals contracting laminitis in the meantime may be too great.

THE NUTRITIONAL VALUE OF PASTURES FOR GROWING AND BREEDING THOROUGHBREDS

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Nutritional strategies for raising foals are generally well understood, principally to provide a ration with adequate levels of minerals and vitamins and optimum amounts of energy and protein for steady growth. However, this can present something of a challenge to breeders, who must attempt to combat the large number of variables that influence the growth of foals. One of these variables is pasture – both in availability and quality. In this work we aim to determine the seasonal variations in nutrient content of pastures, and demonstrate how this relates to broodmare and growing foal requirements.

Pasture samples were collected from three paddock types at a NSW Thoroughbred stud farm over a two year period (Jan 2008 – Jan 2010) and analysed by wet chemistry analysis (Dairy One Forage Testing Laboratory, USA). Calculated digestible energy, and analysed crude protein and mineral levels (Ca, P, Mg, K, Na, Fe, Zn, Cu, Mn) for each paddock type were determined relative to weather observations. Calculated digestible energy, crude protein, calcium, phosphorus, zinc and copper levels of each paddock type were also compared to broodmare (500kg body weight) and weanling/yearling nutrient requirements (based on expected mature weight of 500kg) as recommended by the National Research Council (2007) at each given time point.

A seasonal variation in calculated digestible energy, crude protein, phosphorus, potassium, and zinc levels was observed across all paddock types. More subtle changes were noted for other minerals. On this farm (given adequate pasture availability) the calculated energy levels of each paddock type analysed met, and were in excess of documented broodmare requirements during gestation, but not during lactation. Calculated energy levels were however lower than that recommended for a weanling/yearling at most time points. Crude protein and phosphorus content of all pastures was adequate when compared to broodmare and growing foal requirements all year round. Calcium levels were adequate all year round for broodmares, but in only one of the paddock types for yearlings between July 2008 and March 2009. Levels of the trace minerals zinc and copper in pastures were below that recommended for both broodmares and growing foals all year round.

This study identifies the potential limitations of pastures as a source of nutrients for breeding and growing Thoroughbreds. On-going monitoring of nutrient pasture data within a farm and the development of a farm pasture database offers valuable information for stud managers on pasture deficiencies allowing for the accurate formulation and planning of supplemental feeding programs. Furthermore, information on specific paddock types may be useful when planning the movement of stock around a farm. Taken together, this knowledge may assist in minimising feed costs, while ensuring optimal nutrient intake of growing and breeding horses.

IDENTIFICATION OF HORSES WITH RESISTANCE TO SMALL STRONGYLE INFECTIONS

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This project was a pilot study that investigated the feasibility of developing a simple procedure (or procedures) for the identification of an individual horse's resistance to cyathostomin infections and/or contribution to pasture contamination with nematodes. Such a test/s would facilitate the development of more rational nematode control strategies by identifying those animals that may usefully be targeted for more regular anthelmintic treatments. This offers significant economic advantages (through less anthelmintic usage) as well as welfare benefits (by prolonging the useful life of the only remaining effective anthelmintic group/s) and, potentially, environmental benefits to the horse industry.

Evidence was collected that supports the hypothesis that a small number of horses contribute disproportionately fewer eggs to pasture contamination with cyathostomins. If these horses could be reliably identified with a single test, they could be treated less regularly with anthelmintics than the remainder of the herd and this would lead to cost savings and a reduction in selection pressure for anthelmintic resistance in cyathostomin populations on these properties. Conversely if horses could be identified that regularly contribute disproportionately more eggs to pasture contamination, then these animals could be the focus of worm control strategies. This study found that stallions and ponies were likely to contaminate pastures more heavily (i.e. have higher faecal egg count; [FEC]) than geldings/mares or other breeds respectively, suggesting that this needs to be factored into the design of integrated worm control programs for horse properties.

The study found little correlation between luminal worm burden and FEC, although FEC still reflects a horse's contamination of pasture with parasites. Serum from some horses possesses an agent with anthelmintic activity as measured in *in vitro* larval migration assays, but it appears unlikely that the anthelmintic activity present in the serum is correlated with ability to withstand worm burdens or FEC. However, further work, including with younger animals, is required to confirm this. There was some evidence that MHC haplotype may be correlated with consistently lower FEC, but repeating this work using larger sample sizes is required to confirm this.

Further investigations into the relationships between levels of pasture contamination, serum anthelmintic activity and MHC haplotype are warranted.

USE OF FUNGAL SPORES (*Duddingtonia flagrans*) FOR BIOLOGICAL CONTROL OF INFECTIVE EQUINE INTESTINAL NEMATODE LARVAE

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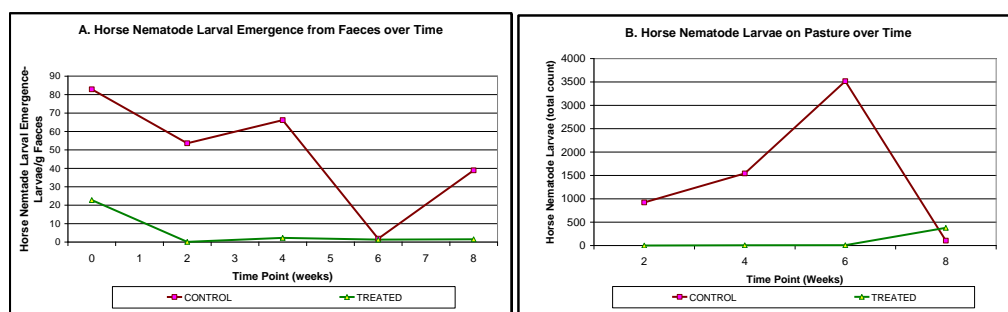
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The natural fungus *Duddingtonia flagrans* belongs to a group of nematophagous fungi that physically entrap nematode larvae by means of adhesive hyphal nets before paralysing and consuming them. When the chlamydospores of *D. flagrans* are fed to grazing animals they pass through the digestive tract without germinating and are subsequently deposited in the faeces along with the eggs shed by adult intestinal parasites. The spores germinate within the faecal pats to produce mycelia with hyphal traps which act to reduce the number of emergent parasite larvae able to migrate to the pasture and re-infect grazing animals (Waller and Faedo, 1996). It should be noted that *Duddingtonia flagrans* is a common soil organism found throughout the world. Studies have shown that spores deposited in faecal pats remain localised and have no significant impact on non-target nematodes and other soil fauna (Knox et al., 2002).

Controlled Australian field trials with grazing animals (sheep, goats, cattle and horses) have shown that use of prototype products containing *D. flagrans* spores can substantially reduce the infectivity of pasture (e.g. Knox and Faedo, 2001). In recently conducted equine field studies, a native Australian strain isolated by CSIRO was used. In the studies, faecal pats from both *D. flagrans*-supplemented and control horses were monitored and it was found (Figure 1) that the use of spores substantially reduced (A) the emergence of nematode larvae within the fecal pats and (B) the infectivity of the pasture surrounding the faeces.

Figure 1 Effect of supplementation with *D. flagrans* spores on (A) larval emergence from faeces and (B) infectivity of pasture



As in previous studies with grazing animals (e.g. Knox and Faedo, 2001), the current results demonstrate that biological control of equine intestinal parasites is a strategy worthy of further study. Importantly, the spores can be administered by mixing into any palatable feed, premix or supplement. Ideally, the horses would be wormed with an effective anthelmintic and moved onto rested pasture where they would be fed the spores during periods of high worm transmission.

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CLIMATE CHANGE AND THE AUSTRALIAN HORSE RACING INDUSTRY

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The world's climate is changing. Global warming, alterations in regional weather patterns and increased frequency of severe weather events combined with an expanding and demanding human population are threatening the sustainability of various animal industries, including the numerous equine industry branches. Limitations on land, water, feed and the ecological changes that result from climatic change will force governments and industries to take proactive or reactive measures to enable adaptation and mitigation for the good of industries and the communities at large into the 21st century. As the Australian Horse Racing Industry is not immune to the potential deleterious effects of climate change, proactive measures need to be instigated in order to protect and maintain it in the face of the challenge.

The initial steps in designing mitigation and adaptation strategies are to assess the specific vulnerability of the industry to climate change. Climate change threats include changes in infectious and non-infectious disease patterns. Specific threats include the likely increase in insect-borne diseases in Southern Australia, an increased likelihood of wildlife-borne diseases as a result of environmental encroachment, and increases in respiratory diseases and potentially orthopaedic and developmental diseases as land becomes barren and nutrient deficient. Increased risks of weather extremes and limitations on available land and water usage may negatively impact upon the industry through losses in training days, heat stress and suboptimal track maintenance, all factors that may deleteriously impact upon the athletic performance of the horse and the viability of race meetings. Seasonal changes may also impact upon breeding and fertility; although warmer condition should enhance fertility this may be counteracted by extreme weather stressors and potential limitations on feed and water. The impact upon breeding may be further compounded in the Thoroughbred industry by the tightly regulated breeding seasons.

In light of government driven climate change mitigation strategies aimed at minimising carbon emission through carbon trading and tax schemes, it's important that the racing industries carbon footprint is evaluated promptly. The emissions related to stud farms, racing stables and racing meeting need to be evaluated by the industry. Activities such as international horse travel by air for both breeding and racing purposes may be contributing significantly to the industry's carbon emission and may attract attention from government and public climate change lobbyists, given that modifications may result in immediate climate change mitigation benefits. The industry needs to be prepared to tackle, modify or justify activities which may be seen as significantly contributors to carbon emission. Evaluating the industry's carbon footprint and the activities within that contribute to it is an important step in facilitating industry justified rather than public preserved climate change mitigation strategies.

Ultimately, the Australian Horse racing industry will be forced to adapt to climate change or mitigate its activities for the public good. This will occur either as a result of internal educated decisions based on scientific and economic evaluation of activities and vulnerabilities with the desired outcomes benefiting both our racing horses and the community, or in response to external government and public forces. The industry needs to decide whether its response is proactive or reactive, with a proactive response starting with industry participants acknowledging an awareness of climate change and the need to tackle climate change issues as an integral component of a sustainable and prosperous Australian racing industry into the 22nd century.

BIOSECURITY RISK MANAGEMENT FOR HORSE PROPERTIES AND EVENTS

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Hendra virus (HeV) is a rare disease with life threatening consequences for horses and humans. This presentation will cover biosecurity measures that are important for all horse owners and event organisers. The measures outlined will not only help to minimise risks of exposure to HeV but also will serve to minimise the risk of spreading a range of other infectious diseases of concern for horses and people.

Biosecurity is the commonsense and practical management of your horses health, your property's health and above all your health. With sensible management practices you can reduce the risk of disease infecting your animals, weeds affecting your property and avoid putting your own health at risk - after all for the majority of horse owners, the welfare of your animals depends on YOU!

A great deal of detailed information concerning HeV and about biosecurity measures for infectious diseases can be found on the Biosecurity Queensland website (<http://www.dpi.qld.gov.au>) and on the Queensland Horse Council website (<http://www.qldhorsecouncil.com>). Please use these websites to learn more about biosecurity and risk management for you and your horse.

Some of the practices will apply to larger establishments, some to the single horse owner. It is your decision how many precautions you take, but even simple changes can save you a lot of money in disease prevention and help prevent both you and your horse from a range of diseases including HeV.

MANAGING HENDRA RISKS – CHANGING BEHAVIOUR

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Hendra is an endemic disease in Australia with low probability of occurrence and extreme consequences for horses and humans.

The clinical signs in horses are non-specific particularly in the early stages of disease. There may not be a typical clinical presentation in infected horses.

While Hendra-infected horses that are severely ill or recently dead present the highest risk of exposure to other horses and humans, infected horses may also be infectious to people and other horses while in the latter stages of incubation, before they show any signs of disease.

Most of the horses known to have been infected with Hendra virus to date and all of the people who have been infected, were exposed and infected before Hendra virus had been confirmed as a diagnosis and therefore before the involvement of authorities in managing response activities.

The major uncontrolled exposure risks to horses and humans are therefore occurring in the day-to-day environment of horse owners and veterinarians dealing with horses as part of their routine activities.

A primary objective for future encounters with Hendra is to minimise the risk of exposure of humans and other animals to the virus.

The most effective precautionary principle involves all people who interact with horses, learning to adopt risk management strategies based on taking precautions even where Hendra virus may not be considered likely to be involved and especially where there is any increase in likelihood of Hendra virus exposure. This involves identifying risks and implementing appropriate precautionary measures before there is any opportunity for exposure to potentially infectious material.

Routine precautions should include measures such as personal hygiene, personal protective equipment (PPE), disinfection and modification of the way people manage their interactions with horses.

This requires behavioural change on the part of everyone who works with horses.

More information can be found on the Queensland Primary Industries web site:

http://www.dpi.qld.gov.au/4790_2900.htm

DEVELOPMENT OF A THOROUGHBRED RACEHORSE WELFARE EDUCATIONAL AND ASSESSMENT PROGRAMME FOR YOUTH GROUPS

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Our aim is to develop an educational programme, for use with equine youth groups, that broadens knowledge of Thoroughbred racehorse welfare requirements and equine welfare. Various National and State Associations, linked to racing, have been invited to send up to two delegates to the first stakeholder meeting at the University of Queensland to determine key welfare issues, which they consider may have negative equine welfare (EW) implications. A Welfare Index (WI) will emerge from the surveys and a range of other group communication methods, including vignettes to contextualise issues. These vignettes will be informed by the Five Freedoms and formulated in a non-threatening manner, intended to prompt an adequate survey response. Respondents will not be chosen randomly but rather on prior knowledge of their awareness of the subject. The Adaptive Conjoint Analysis (ACA) system will be used to estimate final utility values and rank the importance of each issue. Further analysis of data, using a General Linear Model, will test the statistical significance of predicted specific differences and encourage inclusive rather than imposed government policy solutions.

The issues identified at the first meeting are pivotal in developing the framework for an assessment by those involved in the industry of the importance of different welfare issues. Increased awareness of advances in equine scientific methods and the ability of people to make ethical decisions related to EW can be quantified by testing EW knowledge before and after viewing the scenarios on the CD-ROM.

Horse welfare and human safety: Importance of learning, training, and education.

The development of this educational and assessment programme will help youth groups in their understanding of key welfare issues.

DEVELOPMENTAL ORTHOPAEDIC DISEASE IN THOROUGHBRED FOALS: AN EPIDEMIOLOGICAL COMPARISON BETWEEN IRELAND AND AUSTRALIA

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Developmental Orthopaedic Disease (DOD) describes problems affecting the limbs of young horses, including abnormal bone, joint and tendon development. DOD is responsible for major economic losses in the Thoroughbred industry.

Objectives:

- a) Establish the incidence of DOD on a stud in Australia and to compare this with similar data for a stud in Ireland
- b) To determine relationships between factors affecting severity and incidence of DOD in foals with respect to the country in which they are bred and raised
- c) To identify further risk factors associated with the development of DOD

Records of 1717 mares from a major stud in Ireland and another in Australia were made available. Foal weight, age of mare, condition of mare, foal sire and date of birth were monitored over two years. The occurrence of DOD was recorded against these data.

The incidence of DOD was found to be higher on the stud in Australia (average 50%) than on the stud in Ireland (average 14%). Foal weight was found to be a significant factor affecting DOD, with heavier foals showing a proportionally higher severity of the problem. The Australian stud had a higher incidence of DOD in 2000 compared to 1999 (65% affected vs 32% in 1999), whereas the Irish stud had a lower incidence in 1999 compared to 2000 (12% affected vs 16% in 2000). .

The incidence of DOD on one large farm in Ireland was found to be low and stable relative to the large stud farm in Australia. This is a significant finding as the genetic background of the foals on both farms share similarities as some resident stallions shuttle between hemispheres and sire foals on both farms. The dramatic increase in the incidence of DOD in Australian foals over the 1999-2000 period reflects the severity of this major industry problem as well as a greater awareness of the problem

The study provides an excellent basis for further comparative studies investigating DOD in foals with a similar genetic background, but subjected to differing environmental conditions. Large scale, long term studies should be undertaken in Australia that investigate comparative aspects of foal development between Ireland and Australia, including management, husbandry and nutrition.

GENETIC INFLUENCES ON HEART SIZE AND RHYTHM IN HORSES

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Whilst the horse genome has been published, information linking genotype to particular equine phenotypes is lacking. There is a current belief that the abnormally large heart sizes (cardiac hypertrophy) seen in some Thoroughbred horses, and Thoroughbred crosses, may contribute to racing performance. This increased heart size is also associated with predisposition to cardiac arrhythmia.

Preliminary pedigree analysis suggests that the larger than average heart and altered ECG observed in some racehorses may be a familial and/or breed-specific trait and thus have a genetic component.

Orthologous genes that have been conserved across vertebrate and invertebrate species have been shown to play crucial, equivalent roles in determining cardiac rhythm and growth.

In humans, inherited predisposition to cardiac arrhythmia is due to mutations in the genes that encode and control cardiac ion channels while inherited cardiac hypertrophy is caused by mutations in cardiac structural and contractile protein genes.

We hypothesise that the increased cardiac size and altered ECG seen in many Thoroughbred horses is due to mutations in the orthologous equine genes that encode cardiac muscle proteins and ion channels.

We propose to test this hypothesis by cloning and sequencing the equine orthologs of human genes associated with cardiac electrical and contractile regulation, arrhythmias and hypertrophy.

INDUCTION OF ANAESTHESIA IN HORSES WITH ALFAXALONE IN CYCLODEXTRIN

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Horses anaesthetized with alfaxalone with alphadolone in polyoxyethylated castor oil (Saffan) exhibited violent paddling/galloping movements on recovery from anaesthesia (Hall, 1972). A novel formulation of alfaxalone in 2-hydroxypropylbetacyclodextrin (Alfaxan-CD) is available in Australia for use in cats and dogs. We report the results of a randomized, blinded, cross-over trial in which horses received each of ketamine and alfaxalone (Alfaxan-CD) for induction of anaesthesia.

Six horses were randomly assigned to receive either alfaxalone or ketamine as the first of two anaesthetics. Premedication was with xylazine 0.5mg/kg IV followed by 35mg/kg guaifenesin IV. Induction was with alfaxalone 1mg/kg or ketamine 2.2mg/kg. Direct blood pressure, arterial blood gases, heart rate, respiratory rate, time to waking, sternal recumbency and standing were recorded at 5min intervals. Paired t-tests were used to compare between-drug data. Within-drug data were subjected to one-way ANOVA

Two horses swallowed on intubation after ketamine. Good conditions for intubation were recorded for all other anaesthetics. There was no difference in the times from induction to consciousness (ketamine 17.3±1.8min, alfaxalone 17.7±4.1min) or sternal recumbency (ketamine 20.3±3.5min, alfaxalone 25.2±7.7min), but horses took longer to stand after alfaxalone (ketamine 23.1±5.7min, alfaxalone 30.4±7.6min). There were no differences between the two drugs for systolic, mean and diastolic blood pressures or heart rates at each recording time, but horses showed a significant drop in blood pressure with respect to baseline values after alfaxalone. There were no differences in PO₂ between the two drugs at recording times, although both drugs resulted in a significant drop with respect to baseline values. CO₂ was significantly higher after alfaxalone at 10 (alfaxalone 50.8±5.1mmHg ketamine 44.6±7.7mmHg) and 15min (alfaxalone 50.8±4.5mmHg ketamine 45.2±4.5mmHg). There was a significant rise in blood lactate after both alfaxalone and ketamine. Some forelimb rigidity was reported in 5/6 alfaxalone and 2/6 ketamine anaesthetics and tremors in 1/6 ketamine and 2/6 alfaxalone anaesthetics. Recoveries were satisfactory in 4/6 of each anaesthetic agent.

Alfaxalone 1mg/kg IV was comparable with ketamine 2.2mg/kg IV for induction in horses after xylazine 0.5mg/kg and guaifenesin 35mg/kg. After alfaxalone, horses had higher ET CO₂ readings at times during recumbency. Horses took longer to stand after alfaxalone.

The study was funded by the RIRDC Horse Program

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MELANOMA AND THE GREYING HORSE

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Grey horses may be born any colour but, with increasing age, their coats become grey and then white; they could be more accurately described as "greying" horses. Concurrent with this change in coat colour, melanotic tumours frequently develop and these are commonly referred to as grey horse melanomas. Recent identification of the gene responsible for the grey phenotype in horses has renewed interest in this extremely common but poorly understood condition. A consistent theme raised in recent discussions of the condition is the potential for the study of the equine condition as a model for improving our understanding of malignant melanoma in people. However, as has been discussed for over 100 years, there are many aspects of the biology of grey horse melanoma which sets these tumours apart from classically malignant neoplasms such as malignant melanoma.

Although numerous investigations of grey horse melanoma have been published, nearly all of these have relied upon the identification of lesions in live horses. This paper reports the finding of a necropsy-based survey of melanotic lesions in 70 grey horses of varying age and with different levels of carcass involvement. By examining horses with varying severity of disease, it was possible to construct a picture of the likely pattern of EMD progression. This differs from the pattern most commonly described in the literature, in that the usual pattern appears to be slow growth of skin lesions for several years with *concurrent* slow growth of internal lesions. Grey horse melanoma appears to have multicentric origins. The area of the body most consistently involved is the dermis of the distal ventral tail; with time the remainder of the tail, the perineum and the mane also become involved. As tail lesions develop, lesions also develop in connective tissue supporting structures of the parotid region and various axial muscle groups. Lesions also develop in the dermis of the lips, on the fascia of the face, on abdominal fat or serosal surfaces, and within the adventitial layer of major blood vessels. Lymph node involvement is not apparent until quite late in the course of the disease. By the time lesions are clinically detectable dermal masses, numerous lesions are present.

These patterns of carcass involvement are unusual if grey horse melanoma represents a typical malignant neoplasm because dissemination of neoplastic cells via the blood, lymph or direct extension cannot readily explain the distribution of lesions with minimal carcass involvement. This study provided compelling evidence linking grey horse melanoma predilection sites to organs derived from neural crest tissues or in sites known to form part of neural crest migration pathways. Pigment cells are derived from the neural crest and migrate to the skin during embryonic development. This supports a hypothesis that early lesions of grey horse melanoma represent "activation" or differentiation of neural crest-derived cells that have failed to complete embryonic migration.

PRZEWALSKI HORSE CASE STUDY: DEATH RELATED TO EQUINE HERPESVIRUS 1-LIKE VIRUS.

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Equine herpesvirus 1 (EHV1) is a respiratory pathogen of horses that has also been isolated from wild equids, including zebra. The closely related Equine herpesvirus 9 (EHV9) has been isolated from both equid and non-equid captive animals such as gazelle and giraffe. In March 2008 an 8-year old female Przewalski horse (*Equus ferus przewalskii*) at Werribee Open Range Zoo in Melbourne, died following an episode of colic. A series of histological, virological and serological investigations were commenced to definitively identify the causative agent and to investigate the distribution of this agent amongst other populations of captive and wild equids.

Evidence of enteritis was found at post-mortem examination with eosinophilic intranuclear inclusion bodies in the intestinal epithelium and liver, implicating a herpesvirus in the disease process.

A PCR product was amplified from gut and liver using universal herpesvirus primers and sequencing of the amplicon showed close homology to EHV1. This was confirmed after further PCR and sequencing of the glycoprotein G, glycoprotein H and ORF30 regions. Nucleotide identities to the corresponding regions of EHV1 ranged between 96-98%.

The seroprevalence of EHV1 in domesticated horses in Australia has been reported to range from approximately 10-30%. Investigation of the seroprevalence of equine herpesviruses in captive exotic equid populations in 3 Australian zoos found that 80-100% of the sampled captive zebras, but only 12-18% of the captive Przewalski horses tested had antibodies to an EHV1-like virus. This contrasted with serum samples from wild zebra in South Africa where none of the zebras tested were antibody positive. Two of the Przewalski horses at the Werribee Open Range Zoo seroconverted to EHV1 during the study.

EHV1 enterocolitis has rarely been reported in horses. While several reports of herpesvirus disease of captive exotic equids causing severe neurological and respiratory disease have been published, this is the first report of an EHV1-like virus associated with enteric disease in captive equids. Serological studies suggest that EHV1 infection may be more prevalent in captive equid groups than in wild populations, although further verification using larger sample sizes is required.

QUALITY ASSURANCE IN THE RACING INDUSTRY

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“Stewards Investigate Positive Swab” is an all too common headline and is a bane of racing the industry. Positive swabs on race winners attract headlines because they engender within the punting public a sense of an un-fair playing field. Such negative publicity may effect gambling behaviour and in turn reduce gambling turnover. Wagering is the most important financial aspect of the racing industry. Nevertheless trainers continue to present horses to races with drug and metabolite levels above the industry set thresholds. Not only is a positive test damaging to the industry but it is also to the trainers finances and reputation,. A December 2006 Racing Victoria Limited (RVL) media release, reported that a joint operation between the Racing Integrity Department and Veterinary Services achieved the objective of increased confidence and trust in the integrity Victoria racing over the Melbourne Cup carnival. Figures released highlighted the RVL strategy and included no horse testing positive, horses under guard returning an average TCO₂ reading of 31.89mmol, and unauthorized or poorly marked substances uncovered during stable visits dropped from 67 to four. Whilst this result was good, the practice is expensive and only targets the major, high profile racing carnivals.

In an endeavour to maintain the integrity of racing, the industry conducts numerous and regular blood and/or urine sampling of racehorses which normally include all race place getters and systematic screening of out of competition racehorses. This practice is an attempt to create an atmosphere of control and authority to ensure trainers do not use performance enhancing drugs and correctly administer therapeutic drugs prior to racing. To further increase compliance, the Australian Racing Board (ARB) passed amendments to the rules of racing (June, 2009) which placed an obligation on trainers to keep records of any treatment administered to a horse in the trainers care. This rule change is a step in the direction of better control and could be seen as the first part of a quality assurance system for the management of the issue.

Quality Assurance Management systems are common in engineering and manufacturing and are often referred to as process control. As early as World War II systems were developed for production process monitoring because traditional testing wasn't efficient. For example, to test an artillery shells it had to be exploded, to ensure it worked therefore defeating the purpose. These systems were expanded to other manufacturing processes to ensure end product compliance to a standard. Then in the 1960's this approach was adopted by NASA when it commissioned engineers to design and manufacture the first foods for space flights. The resulting systems gained significant popularity. Since then, Quality Assurance Systems have been recognised internationally as a logical tool for adapting traditional inspection methods to a modern, science-based, food safety system based on risk-assessment. These systems allow both industry and government to allocate their resources efficiently in establishing and auditing production practices. Other spin off systems include the ISO 9000 which is a series of standards for quality management systems maintained by the International Organization for Standardization (ISO) and is administered by accreditation and certification bodies. These systems have been applied to a diverse range of businesses and industries where risks must be management, including food safety, environmental management and occupational health and medical systems.

Quality systems have been successfully applied to the beef, dairy, cropping, horticulture, fish farming and fishing industries where there is a diverse range of farming enterprises. There is a need for the racing industry to follow this lead. The racing industry is not unfamiliar with management systems having recently introduced occupational health and safety processes based on risk assessments. Racing stables cover a diverse range from 1 horse stables in outback Queensland to multi-million dollar enterprises with hundreds of horses under care, a scenario is not dissimilar to agriculture.

There is therefore a need to develop and trial quality assurance systems for the racing industry. The appropriate place to start is the management of racing stables. Preliminary research for this project involved interviewing trainers and inspecting stables. In some cases the trainers had been and were still under suspension or had received significant fines for positive swab results. These interviews highlighted significant failures in the stable management processes and in some instances a lack of knowledge. Some trainers had made stupid mistakes and or were careless and unprofessional and had been labelled cheats in the media. Several quality assurance systems have been identified that could be adapted and applied to racing stables to significantly improve management. In agriculture, where farm businesses are often single operator family farms generic quality assurance systems based on an integrated approach with the application of the Hazard Analysis and Critical Control Points (HACCP) approach have been successfully implemented. The research will ultimately make recommendations which will focus on development of a quality assurance system for Australian racing stables. The major benefits and outcome for industry will be better control of an important aspect of racing integrity, provide an opportunity for integrity services to audit or use independent auditors on stable systems, reduced incidents of accidental failures, improved understanding of failures, improved therapeutic drug management and may lead to changes in the way medications are used and allowed to be used.

TRAINING AND RACING MILESTONES IN A SUBSET OF STANDARDBRED HORSES

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The number of Standardbred foals born in New Zealand has been progressively declining for a number of years; analysis of industry trends would provide baseline data essential for future studies.

The aim of this study was to describe the typical pattern of training, age at attainment of training and racing milestones, and the impact of these milestones on career profile, in a selected subset of Standardbred foals.

Within the New Zealand Standardbred industry the training milestones of interest that are readily available are age first registered with a trainer, age of first trial, and age of first race. Harness Racing New Zealand provided from their pedigree and performance database the registration and racing records for all foals born in the 2001 breeding season, which contained training and racing records up to the horses' 6-year-old season. After removal of unregistered and imported horses, and horses exported as yearlings there were 3032 horses (1,521 males and 1,511 females) in this study cohort. Data were examined using frequencies, chi-square, and nominal and binomial logistic regression.

Of the foal crop of 3032, first registration with a trainer was most frequently as 2-year-olds (33.6%), and then as 3-year-olds (25.5%); 31.7% were never registered with a trainer, and 40.5% of horses never trialled. Less than half the foals had entered a race by the end of their 6-year-old career (1448 vs. 1584, $P=0.014$). Horses that raced as 2- ($\chi^2=591.2$, $P<0.001$), 3- ($\chi^2=627.8$, $P<0.001$), or 4-year-olds ($\chi^2=225.4$, $P<0.001$) were more likely to have been registered with a trainer as a 2-year-old than those registered later. Fewer females than males (colts and geldings) were registered with a trainer as 2- ($\chi^2=13.8$, $P<0.001$) and 3-year-olds ($\chi^2=15.6$, $P<0.001$). Fewer females than males started in a race ($\chi^2=31.9$, $P<0.001$), across all age categories.

Over half of the study cohort never started in a race, although two-thirds of the cohort were registered with a trainer at some time. Females were less likely to race than males within each age group. Females were under-represented at the first milestone thus indicating decisions to enter training were heavily influenced by the sex of horse, due to the perceived less attractive racing prospects of fillies.