Equine Science Update

Monthly reports from the world of equine research

May 2002

Early test for ragwort poisoning.

A multi-disciplinary research group at the University of Liverpool Veterinary School is developing a blood test to detect whether a horse is ingesting any ragwort. The test can therefore be regarded as an early indicator of ragwort poisoning long before any clinical signs develop.

Ragwort presents a growing threat to the UK horse population. Dr Derek Knottenbelt of the Liverpool University Veterinary School estimates that around 500 horses and ponies died of liver failure due to ragwort poisoning last year. With the increasingly widespread distribution of the plant, he suggests that this year the total number of equine deaths from ragwort poisoning may reach 1000. This is possibly an underestimate of the problem because the signs are not specific and many cases of ragwort poisoning are not diagnosed.

The toxic effects of ragwort are due to pyrrolizidine alkaloids (PAs) which are present in all parts of the plant, including the seeds.Although PAs also occur in other plants, common ragwort presents the greatest risk for horses because of its widespread distribution in poorly maintained pasture and in grassland used for hay production.

The PAs are rapidly absorbed from the gastro-intestinal tract. They pass to the liver where they are broken down to produce toxic compounds called pyrroles. These act on the DNA of hepatocytes (liver cells) and prevent cell division. Consequently, the hepatocytes are unable to divide and become large cells or "megalocytes." When the megalocytes die they release toxins which may be taken up by neighbouring cells. The dead cells are replaced by fibrosis, which may itself cause further damage to other cells. Thus the damage to the liver is progressive.

The liver is able to maintain normal function until at least two-thirds of its tissue has been destroyed. So the ragwort toxins are able to wreak havoc in the liver without being detected. By the time the horse shows signs of liver failure the damage to the liver is so extensive that treatment is not possible.

Because the liver has many functions, the signs that are seen when it fails vary. The first indications may be weight loss, photo-sensitive dermatitis (inflammation affecting only the non-pigmented skin), lethargy or change in behaviour. As the condition deteriorates, the behavioural abnormalities get worse, the horse starts to wander aimlessly and stagger about. It may stand with its head pressed against the wall, become blind and have difficulty breathing. The signs can develop so quickly that the cause of death may be wrongly attributed to something else like "heart attack", "stroke" or colic. In some cases the horse may be found dead without warning.

Currently there are no simple tests available to specifically detect ragwort poisoning. There are tests that can detect damage to the liver - in particular looking at liver enzymes in the blood. Liver function can be assessed by measuring bile acids. If the liver function is impaired the levels of bile acids in the

Continued overleaf

Contents

Continued from page 1

blood increase. Neither enzymes nor bile acids are specific for ragwort.

It can also be difficult to confirm that ragwort is the cause of the liver disease because the effects of the toxins are seen long after the plant has been ingested. There may be no sign of ragwort in the diet of the horse when signs are seen. A diagnosis of ragwort poisoning may have to rely on finding typical microscopic changes in the liver.

In the absence of an effective treatment for ragwort poisoning it is all the more important to prevent horses having access to the plant either at pasture or in contaminated hay. Preventing ingestion of ragwort is the only way to prevent ragwort poisoning.

Ragwort can be difficult to identify when it is dry in hay. Feed samples can be analysed for PA content. This is time consuming, relatively expensive, and the reliability of the result depends upon the accuracy of the sample.

Clinicians at the Liverpool Vet. School have been working with scientists from the University`s Protein Function Group to develop a test to detect early signs of exposure to ragwort. A pilot study has shown that the test is effective at recognising changes in the blood due to small amounts of pyrrolizidine alkaloids. The test recognises alterations in certain components of the blood cells caused by the toxins. A positive result indicates current or recent access to the toxin in feed.

This simple screening test will make it easier to check whether the food being given is safe or not. Early detection of exposure to ragwort will allow suitable preventative measures to be taken.

According to Dr Knottenbelt the test will provide an easier way of checking that hay is or is not contaminated with ragwort. "We visualise that the test will be used on a random sample of horses in a yard every 6 - 12 weeks or so. If evidence of the toxin is found in any of the samples, the hay or grazing must be examined for ragwort. "

"This test will be a preventative measure that will let owners know their horses are eating ragwort. The affected feed can then be destroyed and appropriate changes in the diet can be made." says Knottenbelt. By stopping access to contaminated food at an early stage, before significant levels of the toxins had been absorbed, it may be possible to prevent irreversible and progressive liver damage.

The development of this test heralds the prospect of reversing the mounting death toll due to ragwort. However, the test is not currently available as more funds are required to validate it for general use. Before the test can be available to vets in practice, further work needs to be done to confirm its accuracy and reliability.

A charity, the Ragwort Trust, has been set up to fund the research. Dr Knottenbelt points out that £80,000 is needed. He would be pleased to receive any donations to enable this important work to continue. Cheques should be made payable to "University of Liverpool" and addressed to Dr Derek Knottenbelt, University of Liverpool, Leahurst, Neston, Wirral, CH64 7TE, with a covering note to state that the donation is for the Ragwort Research Fund.

Liverpool University is a registered Charity, and Gift Aid forms are available from Dr Knottenbelt from the above address (or email: knotty@liv.ac.uk)

Airway inflammation affects dope-tests.

Small airway inflammatory disease (SAID) affects the absorption and excretion of gases in the lungs, and may produce false positive results in dope-tests, according to work carried out recently in Milan.

SAID is a common cause of poor performance in young racehorses. Usually the clinical signs are non-specific. It is often necessary to perform investigations such as a broncho-alveolar lavage (BAL) to confirm the diagnosis. BAL consists of flushing the lower airways with sterile saline and assessing the number and type of cells present in the retrieved sample. This can help identify the type of inflammatory process involved.

Inflammation of the lower airways may interfere with the absorption of oxygen and the excretion of carbon dioxide, leading to an increase in carbon dioxide in the blood ("respiratory acidosis"). This may contribute to the reduction in performance in horses with SAID.

Clinicians at the Institute of Veterinary Internal Medicine, University of Milan assessed fifty-three racehorses with poor performance associated with SAID. They used arterial blood gas analysis to evaluate what effect the SAID had on lung function.

Three quarters of the horses had a cough. Just over half (30 horses) had a mucous or mucopurulent nasal discharge. Endoscopic examination showed signs of inflammation such as varying degrees of hyperplasia of the lymphoid tissue in the throat ("pharyngeal lymphoid hyperplasia"), exudate in the trachea and inflammation of the lower airway.

Broncho-alveolar lavage was used to confirm the diagnosis in all cases including those in which there were no obvious clinical signs. All horses had more cells than normal in the lavage fluid. (In the absence of inflammation the cell count would be less than 300 / µl). There was also an increase in several inflammatory cell types, such as neutrophils, mast cells and eosinophils, confirming the presence of SAID.

Blood gas analysis was used to assess lung function. The blood takes carbon dioxide (CO_2) to the lungs where it is exchanged for oxygen (O_2). As well as being carried in the blood as dissolved gas, some of the CO_2 undergoes an enzyme-controlled reaction with water to produce bicarbonate ions. ($H_2O + CO_2$ $HCO_3^- + H^+$). So the blood contains carbon dioxide in two forms : dissolved (measured as pCO_2) and bicarbonate (measured as HCO_3^-). The oxygen content of the blood is measured as pO_2. If the gas exchange is not adequate, the pCO_2 increases and the pO_2 decreases.

Blood gas analysis showed that 58.5% of the horses had higher levels of CO_2 in the arterial blood than normal ("hypercapnic"). The pO_2 was within the normal range in all horses but tended to be slightly lower in the horses which were hypercapnic.

When the researchers correlated the results of the blood gas analysis with the duration of the SAID, they found that gas exchange was more likely to be impaired in horses that had been affected with SAID for over 4 weeks. Not only did most of those horses have raised pCO_2, they also had raised HCO_3^- and total carbon dioxide (TCO_2). Most horses affected with SAID for less than 4 weeks showed no significant change in arterial blood gases.

Dr Francesco Ferrucci, spokesman for the researchers, points out that the increase in total carbon dioxide is particularly significant in countries where anti-doping controls include the measuring of total CO_2 content of the blood (as a means of detecting fraudulent administration of bicarbonate to improve racing performance). He suggests that an increase in TCO_2 could arise as a result of sub-clinical SAID. Further tests, such as BAL, might be necessary to rule out the possibility that the increase in TCO_2 is due to SAID before sanctioning a competitor for fraudulent bicarbonate administration.

The results showed that, unlike recurrent airway obstruction (RAO or COPD) in which both the pCO_2 is increased and the pO_2 deceased, with SAID the changes are more mild, affecting only the carbon dioxide. In fact most of the horses affected for less than 4 weeks had values of pCO_2 that fell within the normal range.

for more details see

Arterial blood gas analysis in 53 racehorses with a diagnosis of small airway inflammatory disease (SAID) Elisabetta Ferro, Francesco Ferruci, Enrica Zucca, Viviana Di Fabio, Sabrina Castoldi
J Eq Vet Sci (2002) 22, 165 - 168

New treatment for suspensory ligament strains

A more effective method for treating strains of the suspensory ligament has been developed at the Alamo Pintado Equine Medical Center in California.

Suspensory ligament injuries are a major cause of lameness in athletic horses. They present a therapeutic challenge. Suspensory ligaments heal slowly. The healing process often results in poor quality repair tissue which leaves the ligament predisposed to further damage. Repeat injuries are common.

Various techniques have been used to try and improve the quality of repair -including ligament splitting, hyaluronic acid injections, blistering and firing. The results have not been encouraging, and no method has been shown to be effective in critical clinical studies.

Dr Douglas Herthel has developed a technique ("autologous bone marrow injection" or ABMI) in which a sample of the horse`s own bone marrow is injected into the damaged suspensory ligament. He explained the procedure at the 2001 Convention of the American Association of Equine Practitioners.

Bone marrow contains cells, growth factors, (such as platelet derived growth factor and transforming growth factor) and fibrinogen, which aid the healing of damaged tissue.

He carries out the procedure under general anaesthetic, with the horse lying on its back. He places needles into the damaged portion of suspensory ligament (guided by ultrasound scan). He then removes about 30 ml of bone marrow from the sternum (breast-bone) of the same horse and injects it through the pre-placed needles into the ligament. The only side-effects that he noticed in a series of 100 cases was soreness for a couple of days after the procedure in two horses.

Herthel reviewed the results of the first 100 horses treated using this method. He compared the results with 66 horses treated by other means. Of the 100 horses, 81 had suspensory desmitis of the forelimb and 19 involved the hind limb. The injuries most commonly affected dressage, jumping and racing horses.

Of the horses treated with ABMI, 84% became sound within 6 months and returned to work. A further 8% showed some improvement but were not completely sound. In comparison, only 15.2% of cases treated by other means were sound a year after being injured.

By using ultrasound to monitor the healing process, Dr Herthel demonstrated that suspensory ligaments treated with ABMI had improved fibre alignment and a smaller cross-sectional area compared with ligaments which received other treatments. He noticed that the improvement in the ultrasound appearance occurs more rapidly in the ABMI-treated suspensory ligaments.

Dr Herthel has now treated over 400 cases of tendon and ligament damage using this technique and recommends it for the treatment of horses with suspensory ligament desmitis.

"We have used this novel biological method to encourage suspensory ligament healing for the past six years. The rate of return to soundness is significantly higher than reported for other techniques."

" The use of stem cells and associated bone marrow components result in high-quality suspensory ligament healing, which is a practical, effective, economic, and safe procedure available to veterinarians dealing with sport horse injuries."

For more details see:
Enhanced suspensory ligament healing in 100 horses by stem cells and other bone marrow components. Douglas J Herthel
Proc Am Assoc Equine Pract (2001) 47, 319 - 321

Safer oestrus induction.

A more effective, cheaper technique for bringing mares in season has been described, which, it is claimed, also causes fewer side effects than current methods.

The natural oestrus cycle of the mare is regulated by prostaglandin. If the mare has not conceived, prostaglandin (PGF2) is released from the uterus about 13 - 16 days after ovulation. Prostaglandin causes luteolysis (ie it destroys the corpus luteum, which has been producing progesterone, the hormone responsible for the mare not being in season.)

Prostaglandin is widely used in stud practice to bring the mare in season, and is available as either the natural hormone, PGF2 , or as a synthetic analogue.

Currently prostaglandin is used as a single intramuscular dose. This can produce side effects in a proportion of mares. As many as 10% may show signs of discomfort after injection - such as sweating , diarrhoea or colic. Another problem encountered is that a single dose may not be effective. It has also been shown that a single dose of prostaglandin may fail to induce luteolysis in 7% of mares.

In the natural situation prostaglandin is usually released in several small pulses.

Now scientists at Lincoln University, in Canterbury, New Zealand, have demonstrated that using two small doses of prostaglandin, given twenty-four hours apart, is effective at inducing oestrus and causes fewer side effects than the conventional single large dose.

The researchers, lead by Professor Cliff Irvine, evaluated the response to various different doses of PGF2 in a group of eight mares. They gave all the mares 5mg PGF2 seven days after they had ovulated (ie at the time when they would be expected to be responsive to treatment with prostaglandin.) Then they treated half of the mares with a single dose of 1.5mg and 0.5 mg on separate occasions. To the other half they gave two doses of 0.5mg 24 hours apart. The selected treatment was given about 7 days after each mare had last ovulated. They found that the severity of the side effects (such as sweating and spasms of the pelvic muscles) increased with the dose given.

In a second study, 39 Standardbred mares were treated with prostaglandin. Twenty two were given 10mg PGF2 . The other 17 received two doses of 0.5mg PGF2 24 hours apart. All 17 mares given the two small doses showed signs that the corpus luteum had lysed. The blood progesterone level fell after the first dose but the second dose was required to reduce it to below 0.9ng/ml. The time for luteolysis to occur was the same for the two treatment regimes. Interestingly the researchers found that a single large dose (10mg) of prostaglandin was actually less effective than two 0.5mg doses.

"The most striking observation was that luteolysis in mares was induced more reliably with two 0.5mg doses of PGF2 given intramuscularly 24 hours apart, than with the standard single dose of 5 or 10 mg." reports Prof. Irvine. "We found that the severity of side effects of PGF2 administration increased with dose. Side effects can be minimised by giving two doses of 0.5mg PGF2 24 hours apart."

For more details see:

Effectiveness of a two-dose regimen of prostaglandin administration in inducing luteolysis without adverse side effects in mares.
CHG Irvine, V-L McKeough, JE Turner, SL Alexander, TB Taylor.
Equine Vet J (2002) 191 - 194

Upward fixation of the patella: new treatment.

Upward fixation of the patella is a common condition of the stifle joint. It occurs when the medial patellar ligament hooks over the upper end of the medial trochlea of the femur (one of the two the bony ridges on which the patella slides).

In the most severe cases the patella becomes fixed and the horse is unable to flex the leg. The horse stands with the affected hind leg extended behind it. Less severe cases show partial, intermittent upward fixation, which produces visible, and sometimes audible, clicking as the patella frees itself.

The condition is most common in younger horses, and is often associated with poor muscle conditioning. Horses with straight hind limb conformation are more susceptible than horses with normal conformation. Both hind legs may be affected to some extent. When upward fixation of the patella occurs, the ligament stretches making a recurrence of the condition likely.

In less serious cases increased exercise, to improve muscle tone, may be all that is required to reduce recurrence of the condition. However, persistent cases may require surgical intervention.

For many years the recommended treatment involved cutting the medial patellar ligament ("medial patellar desmotomy"). Although this is effective at preventing the patella becoming fixed, recent work has shown that it is not without adverse effects. Fragmentation of the patella occurs after the operation in a significant number of cases.

Now a new technique has been developed which is effective and does not appear to be associated with side effects. Dr Aziz Tnibar, working in France at the Equine Clinic of the Veterinary School of Alfort, has devised a procedure in which the upper third of the medial patellar ligament is split.

The horse is anaesthetised for the procedure. Dr Tnibar uses ultrasound guidance to ensure the correct placing of the cuts, making sure that they do not extend into the femoro-patella joint or into the fibrocartilage extension of the patella

The aim of the operation is to cause thickening in the ligament to prevent it becoming hooked over the end of the femur.

"The rationale for percutaneous splitting of the upper third of the medial patellar ligament is to induce a localized desmitis, which subsequently leads to a localised thickening of the ligament. This will make it more difficult for the upper part of the ligament to hook over the medial ridge of the femoral trochlea" says Dr Tnibar.

Dr Tnibar has now performed this operation on seven horses and ponies. All seven were affected with upward fixation of the patella in both back legs. After medial patellar ligament splitting, all seven became sound and returned to work. In three horses the upward fixation of the patella resolved completely within 24 hours. In the other four cases, the condition resolved within 24 hours in one leg. The other leg improved gradually over a period of 4 - 12 days.

Dr Tnibar recommends daily light exercise (walking in hand for 15 minutes three times day) for 2 weeks after surgery. Normal activity is gradually introduced after two weeks.

Follow-up examinations show that the upper third of the medial patellar ligament becomes thickened. There is a progressive increase in medial patellar ligament size during the first four weeks after surgery. Radiographic examinations have not shown any sign of damage. In particular, no horses have developed fragmentation of the patella .

Dr Tnibar concludes that this is a successful technique for the treatment of upward fixation of the patella. "In each case all evidence of upward fixation of the patella has disappeared, and the horse (or pony) has regained its normal activity".

For more details see:

Medial patellar ligament splitting for the treatment of upward fixation of the patella in the horse. M Aziz Tnibar *Proc Am Assoc Equine Pract (2001) 47, 491 - 493*

Anti-oxidants and lung function.

Recent work suggests that balanced dietary supplementation with antioxidants may help in the management of respiratory disease in horses.

At a recent meeting at the Animal Health Trust, Dr. David Marlin explained the importance of antioxidants in respiratory disease in the horse.

Reactive oxygen species ("ROS" otherwise known as "free radicals" or "oxidants") play an important part in the inflammatory process. In excess they can cause cell damage, including damage to the DNA, and cell death. They are involved in many diseases of the respiratory tract including asthma, oxygen toxicity, and recurrent airway obstruction (RAO) in horses.

Anti-oxidants, often described as "free radical scavengers", act to limit tissue damage by preventing an accumulation of ROS. The horse has various antioxidative mechanisms including enzymatic systems such as superoxide dismutase and catalase; and non-enzymic mechanisms such as as corbic acid and glutathione.

In the normal animal, oxidants and anti-oxidants are in balance. A change in that balance, with an increase in oxidants ("oxidative stress"), plays an important role in the development of inflammatory airway disease. Oxidative stress may be caused by factors such as disease (allergy, infection); environmental factors (pollution, radiation, physical injury) or diet (high concentrations of polyunsaturated oils).

Antioxidants exist in two forms: reduced (available for action) and oxidised (after being used to neutralise ROS). The ratio between the oxidised antioxidants and the total antioxidants is used as a measure of oxidative stress and is called the "redox ratio". Horses with RAO have much higher redox ratios than normal horses. Treatment of the inflammation in horses with RAO reduces the pulmonary oxidative stress.

Investigations carried out at the Animal Health Trust have shown that ascorbic acid (vitamin C) is the most important antioxidant in the respiratory tract of the horse. In normal horses the concentration of ascorbic acid in the epithelial lining fluid (ELF) of the respiratory tract is very high. In comparison, horses with recurrent airway obstruction (RAO) have a markedly reduced ascorbic acid concentration in the ELF. The concentration of ascorbic acid in the blood is also reduced in horses with RAO. Other antioxidants such as uric acid, vitamin E, and glutathione, appear to be less affected and show no difference between horses with RAO and normal horses.

The proper functioning of the respiratory system is especially important for performance horses. Unlike humans, horses can produce their own vitamin C. But hard work or other stresses may interfere with ascorbic acid production. It may take more than four months for antioxidant levels in the ELF to return to normal following respiratory disease. In some horses they may never return to normal without dietary supplementation.

Researchers at the Animal Health Trust and the Waltham Centre for Pet Nutrition, have been investigating whether lung ascorbic acid levels can be increased by dietary supplementation. In one study they found that dietary supplementation increased the ELF ascorbic acid concentration by about 40% even in apparently healthy horses. However, a single antioxidant added to the diet is not adequate. A balanced mixture is required.

Dr. Marlin pointed out that too much as corbic acid is harmful. He recalled one

Continued overleaf

Continued from page 7

instance of poor performance in a training yard of TB racehorses which was associated with excessive administration of ascorbic acid.

In another study, eight horses, each with a history of RAO but not showing signs at the time, were assessed at rest and after a standard exercise test. Assessments were carried out both before and after four weeks of dietary supplementation with a balanced mixture* of antioxidants. The researchers found significantly improved exercise tolerance and reduced inflammation in the airways. The results suggest that the antioxidant mixture tested improves lung function in horses with RAO, by altering the oxidant/antioxidant balance and reducing airway inflammation.

"Uncontrolled ROS production causes tissue damage and propagates inflammation. There is evidence of oxidative stress and reduced anti-oxidants in RAO, but also in healthy horses during exercise" concluded Dr. Marlin. " A balanced antioxidant supplement may improve lung function in horses with RAO and in healthy horses especially when they are stressed. An adequate antioxidant intake is important for normal lung function and may be protective. The precise form of antioxidant is important."

* based on Winergy Ventiliate ® Waltham Centre for Pet Nutrition

For more details see:

1)**Ascorbic acid in equine plasma and epithelial lining fluid in healthy horses and horses affected by recurrent airway obstruction (RAO)** Smith N, Marlin D, Deaton C, Roberts C, Kelly F, Harris P, Schroter RC (2001) *Proceedings of the World Equine Airways Symposium 35*

2) **Effect of nutritional antioxidant supplementation on systemic and pulmonary antioxidant status, airway inflammation and lung function in heaves-affected horses**. Kirschvink N, Fievez L, Bougnet V, Art T, Degand G, Smith N, Marlin D, Roberts C, Harris P, Lekeux P. (2002) *Equine Vet J (in press)*

Back pain research

Clinicians at the Cambridge University Veterinary School are actively seeking referrals for chronic back and sacro-iliac problems in horses for diagnosis and treatment.

Professor Leo Jeffcott, who heads the Department of Clinical Veterinary Medicine, points out that they have established clinical expertise in back conditions over a number of years and have a full range of facilities to assist in diagnosis.

They are keen to assess the benefit of treatment with shock wave therapy in suitable cases, and would like to see as many cases as possible over the next few months as part of a prospective clinical trial.

Owners of horses with back problems should, in the first instance, contact their own vet who can arrange an appointment with Prof Jeffcott.

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