

JOHNE'S TESTING IN ALPACAS

The recent positive diagnosis of Johne's disease in two alpacas has sparked many enquiries about serological testing for Johne's in alpacas.

Unfortunately, none of the serological tests currently available are designed for use in alpacas and llamas. Many of the ELISA tests appear to have problems with specificity (i.e. animals may be falsely positive). The sensitivity of these tests is also not documented. The Gel Diffusion test has been evaluated in a herd of Johne's free alpacas, and has at least been shown to have good specificity (i.e. it does not produce any false positives). However the sensitivity of this test has not been evaluated.

Other tests can be performed on the live animal and may be useful. Animals that have relatively advanced disease may shed large numbers of acid-fast organisms in their faeces. If present, these are easily detected on staining and examination of the faeces. Unfortunately, not all animals with Johne's will shed the organisms or they may only shed organisms intermittently. So the test, while having excellent specificity, has limited sensitivity especially for one-off samples.

The only test which is considered both sensitive and specific for diagnosis of Johne's pre-mortem in alpacas

and llamas is faecal culture. This is a relatively expensive test and unfortunately results take up to 12 weeks to become available due to the slow growth of the organism.

Screening clinically normal animals using serological tests for Johne's is not currently recommended. Serological testing using approved tests in cattle, sheep, goats and deer is only recommended in animals with consistent clinical signs of wasting and/or chronic scour.

$$\text{Sensitivity} = \frac{\text{The number of animals that test positive}}{\text{The number of animals that actually HAVE the disease}} \times 100\%$$

$$\text{Specificity} = \frac{\text{The number of animals that test negative}}{\text{The number of animals that actually DON'T HAVE the disease}} \times 100\%$$

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References:

Miller, D.S. et al. Specificity of four serologic assays for Mycobacterium avium ss paratuberculosis in llamas and alpacas: a single herd study. *J Vet Diagn Invest* 12:345-353, 2000.

TWO CASES OF ZINC TOXICITY IN CALVES

A 14-week-old calf presented with haematuria. The calf had been given a zinc bullet intended for an adult.



The main abnormalities were in the CBC where there was a moderate anaemia (Hct 0.16 [RR 0.23-0.42]) with evidence of early regeneration and a few Heinz bodies. The latter are seen with the Brassica crops but also are reported in zinc toxicity cases. The serum sample was haemolysed and bilirubin mildly increased.

The serum zinc level was massive at 639 umol/l confirming zinc toxicity as the cause of the haemolysis. Lepto titres for pomona and hardjo were negative. GGT levels were within reference range and spore counts at the time were low, ruling out haemolysis due to facial eczema.

Chelation therapy with EDTA at empirical doses was successful in treating this calf. This therapy is generally used for lead toxicity but will work for other divalent ions. It is actually recommended that zinc supplementation is given when treating lead poisoning as the EDTA is pretty efficient at removing zinc.

In another case of zinc toxicity, one or two deaths occurred in a mob of six-month-old calves with the remainder of the mob experiencing symptoms which included, weakness and a bright green scour. Diagnostic testing ruled out Yersiniosis and a significant worm burden. The selenium status of the calves was marginal but the major finding was of severe serum zinc elevations at 130, 227 and 253 umol/l (reference range: up to 35 umol/l in anticipation of sporidesmin

toxicity). These results confirmed zinc toxicity and this would have been responsible for the bright green diarrhoea clinically. And the cause of this toxicity?? It transpired that the farmer had been over-zealously adding a zinc product directly to the water troughs.

Thanks to Dr James Bosley, Veterinary Associates Equine & Farm, Takanini for the first case and to Dr Scott Raleigh, Barks Corner Veterinary Hospital, Tauranga for the second case.

Jenni Donald and Angus Black

P R O D U C T I O N / E Q U I N E

RENAL TUBULAR ACIDOSIS IN A HORSE

Renal disease in general is rare in horses and renal tubular acidosis (RTA) is one of the least common causes. In this condition there is renal tubular loss of bicarbonate and replacement by chloride. With Type 1 RTA, the distal renal tubule fails to secrete hydrogen ions and in Type 2, the proximal tubule inadequately resorbs bicarbonate. Type 4 RTA is common in humans but has never been reported in horses. This form is associated with hypoaldosteronism or resistance of the distal nephron to the actions of aldosterone.

The causes of RTA are not understood. In humans, this condition can be primary (genetic or idiopathic) or secondary following a variety of conditions such as hyperglobulinemia, autoimmune disease or inflammatory, obstructive or toxic kidney disease. In horses, most have been reported as idiopathic as there has been no evidence of primary kidney, liver or immune mediated disease, no evidence of a disturbance of calcium metabolism and no history of access to toxins.

The clinical signs are listed as anorexia, depression, weight loss and weakness which is how this 2-year-old Warmblood mare presented. She was also PU/PD.

Initial blood tests showed a severe metabolic acidosis with normal sodium, moderate hypokalemia and a moderate hyperchloremia.

The differentials for these changes are:

- GI loss of electrolytes with diarrhoea – no evidence of this on clinical exam
- Renal tubular acidosis – either Type 1 or 2 – most likely diagnosis.

The other changes were a mild decrease in calcium and magnesium (likely from the anorexia), increased creatinine (from renal compromise due to the electrolyte imbalance) and increased GLDH (indicative of acute hepatocellular damage – possibly secondary to the acidosis) - see Table 1.

With RTA in humans, they endeavour to determine whether it is Type 1 or 2 but generally in horses this is not the case as the treatment is the same. Treatment involves replacing bicarb and potassium.

After 10 days bicarb supplementation, there was a marked improvement. The mare was much brighter, there had been weight gain and her appetite was back to normal.

TABLE 1

		Units	Ref Range
CK	688	IU/L	63-469
AST	282	IU/L	0-700
GGT	11	IU/L	7-45
GLDH	89	H IU/L	1-8
Bilirubin	34	umol/L	10-42
Total Protein	67	g/L	57-76
Albumin	32	g/L	32-40
Globulin	35	g/L	20-41
Urea	7.0	mmol/Ll	3.0-9.2
Creatinine	164	H umol/L	97-144
Phosphate	1.15	mmol/L	0.9-1.7
Calcium	2.67	L mmol/L	2.8-3.3
Magnesium	0.62	L mmol/L	0.68-0.90
Sodium	137	mmol/L	131-141
Potassium	2.6	L mmol/L	3.0-4.6
Chloride	110	H mmol/L	94-104
Bicarbonate	11	L mmol/L	26-35

TABLE 2

		Units	Ref Range
Sodium	138	mmol/L	131-141
Potassium	3.3	mmol/L	3.0-4.6
Chloride	102	mmol/L	94-104
Bicarb	20	L mmol/L	26-35

Electrolyte levels were checked – see Table 2.

As to long term prognosis with this condition – quote from Warwick Bayley, Section 17.10, Equine Internal Medicine, "Periodic rechecking of the animal is advised until it becomes clear that its condition is stable. Long term follow-up of cases the author has seen and those that have been reported in the literature suggest that the prognosis is favourable."

Thanks to Dr Rosie Richards, Veterinary Associates, Takanini for this case.

Jenni Donald

References:

•Equine Internal Medicine, Eds. Reed, Bayly & Sellon, 2nd Edition

DERMATOPHILOSIS IN A YOUNG BULL

This yearling bull had generalised seborrhoea and furunculosis involving the whole body. Cytological exam of material from under the scabs had large numbers of inflammatory cells and many bacterial filaments with the typical "railroad track" appearance of *Dermatophilus congolensis*.

Thanks to Celia Grant for this case and the photo from the field.

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SPORIDESMIN TOXICITY – A BAD YEAR?

Facial eczema pasture spore counting does not appear to have been the whole story this year. Although trends in spore count numbers show that they do not in general appear to have been as high as in 2006, average GGT levels in cattle seemed to rise quickly after rain in early and mid March, and in some cases peak to much higher levels than those seen in previous years.

From the rainfall data, there appears to have been a lengthy hot, dry spell through February. Could a relative shortage of feed contributed to an increased sporidesmin load despite the fact that spore counts did not seem any higher than in 2006?

This year we have seen an unusual number of animals presenting with very acute sporidesmin toxicity. In these patients, massive haemolysis occurs, resulting in severe icterus and haemoglobinuria. Death frequently quickly follows. In one classic case, a dairy cow exhibited red water and appeared off colour. This cow had severe haemolytic anaemia with a red blood cell count of $1 \times 10^{12}/L$ (ref range $5 - 7.7 \times 10^{12}/L$). GGT levels in this animal were very elevated, 517 IU/L (ref range 0-36 IU/L). This cow died and histologic examination of her liver revealed marked biliary epithelial proliferation with cholestasis, accompanied by an acute peri-acinar necrosis. The biliary epithelial proliferation occurs in response to the damage caused by the sporidesmin toxin itself in these lesions. The peri-acinar necrosis is due to the severe anaemia and hypoxic damage to the liver. The haemolysis is thought to occur due to direct effects of the sporidesmin toxin on the red blood cell membranes, and as a result of oxidative free radical damage.

In some cases, it appears that sudden exposure to very high levels of sporidesmin causes haemolysis to occur so quickly that death happens before there is histologically evident epithelial damage. On one property on which this occurred, the diagnosis of sporidesmin toxicity was only made after a few days, when other animals on the property began showing cutaneous signs

This past season has also taught us that spore counts are not the whole story when considering sporidesmin exposure. If a dry spell results in a shortage of feed, then increased grazing pressure will certainly influence the levels of exposure, and could result in acute episodes of toxicity such as those described here.

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References:
Meat and Wool New Zealand. Facial eczema risk and incidence monitor no. 16. April 27, 2007.
Upreti, G.C. and Jain, M.K. Interaction of sporidesmin, a mycotoxin from *Pithomyces charatrum*, with lipid bilayers. *Bioscience Reports*, 13(4): 233-243, 1993.
Munday, R. Studies on the mechanism of toxicity of the mycotoxin sporidesmin. 2 – evidence for intracellular generation of superoxide radical from sporidesmin. *Journal of Applied Toxicology* 4(4): 176-181, 1984.

HINTS FOR SUCCESSFUL LARVAL CULTURES

Larval cultures are increasingly being requested as part of faecal egg count reduction testing. Results of larval cultures can help give you an idea of which species of worms may be developing resistance to certain anthelmintics. However, there are certain factors you may want to keep in mind when assessing larval culture results.

Conditions under which faeces are transported and stored can drastically affect larval culture results. Eggs of some species of worms may be more affected by environmental conditions than others. Refrigeration, in particular, has been shown to affect hatchability of worm eggs. In one study, the eggs of *Cooperia* and *Haemonchus* species were shown to be markedly more susceptible to exposure to temperatures of 4 deg C than those of *Trichostrongylus* and *Ostertagia*, with *Oesophagostomum/Chabertia* being most resistant.

The traditional instruction has been to refrigerate faecal egg count specimens. This strategy works well for specimens used for faecal egg counts only as it prevents the eggs from hatching. However, for specimens which may be used subsequently for larval culture it is not such a good idea. It is still necessary for faecal samples to be prevented from exposure to excessive heat, but storage and shipping at ambient temperature can be used provided the samples will arrive at the laboratory within 24 hours.

To facilitate transport and prompt set up of specimens for larval culture, we recommend that samples on which larval culture may be required be submitted for arrival at the lab on a weekday, rather than be 'in transit' over a weekend. Routine parasitology is not done over the weekends, so samples intended for larval culture should not be timed to arrive at the lab on Saturdays.

EQUINE IGG TESTS

Foaling season is just around the corner and it's a good opportunity to review the options available for IgG testing. NZVP offers both screening and quantitative tests for determining foal IgG status.

IgG Screen Test: Is also commonly referred to as Glutaraldehyde (Coagulation) Test. The IgG Screen test is not specific for equine IgG. The glutaraldehyde reacts with proteins in the serum, most of which are IgG's, thus providing an estimate of the IgG level in the serum. In most cases, this test will provide a reasonably accurate semi-quantitative IgG result within 2-3 hours of receipt at the laboratory. However, it is important to note that in some cases where the foal is unwell (e.g. bacterial infection, septicaemia), there may be increased level of proteins in the serum which can result in erroneously high IgG levels, due to the glutaraldehyde reacting with all proteins in the serum. In these cases, a TIA test would be recommended.

IgG TIA Test: TIA (or turbidimetric immunoassay) is an equine IgG-specific quantitative test that was first launched on to the NZ veterinary diagnostic market by NZVP Ltd in 2006. This is an

It is important that we know as soon as possible after faecal egg count results are issued if you would like us to set up a larval culture. This reduces the storage time for faeces and helps ensure good larval recovery. If you are not sure whether you will be about to see results, you can always ask us to do larval culture contingent on faecal egg count results.

Important points to remember when submitting samples for larval culture:

- 1) Fresh samples can be stored and shipped within 24 hours at ambient temperatures. Do not allow samples to become overheated. Samples which have been held too long at ambient temperature will hatch and not be useful for FEC.
- 2) Ship Mondays – Thursdays. Do not ship overnight on Fridays or over the weekend.
- 3) Samples should be submitted in moisture proof containers so that they do not dry out. Pottles or sealed plastic bags are best.

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References:
Berrie, DA et al. Differential recoveries from faecal cultures of some gastro-intestinal nematodes of cattle. *J Helminthol*. 1988 Jun; 62(2): 110-114.
McKenna, PB. The effect of previous cold storage on the subsequent recovery of infective third stage nematode larvae from sheep faeces. *Vet Parasitol*. 1998 Dec 31; 80(2): 167-172.

automated test performed on the Biochemistry analyser which provides a quantitative test result within 2-3 hours of receipt at the laboratory. The TIA test provides more consistent and accurate results in a quicker turnaround time than the RID test, which is a manual test that can take 18-24 hours to get a result. The TIA test was validated against the RID test with excellent correlation and the TIA results are accepted by most bloodstock companies for insurance purposes. If you would like more information about this test, please contact the laboratory.

IgG RID Test: This test is still available but is being phased out in favour of the TIA test. This is an equine IgG-specific quantitative test which is set up in a gel and run overnight. The sample precipitation rings are measured and compared back to a standard curve to determine the IgG result. This test is a completely manual assay and prone to variation due to manufacturing, technical and environmental factors. The turnaround time is 18 – 24 hours.

If you have any questions regarding the various equine IgG tests please do not hesitate to contact the Serology Dept.