

TESTING UPDATES

BRUCELLA OVIS

We are seeing quite a few *Brucella ovis* samples coming through the laboratory. *B. ovis* monitoring is part of a national scheme and the testing is performed by one laboratory (Gribbles, Palmerston North) for all NZ samples. *B. ovis* CFT test for the NZ Scheme costs \$XXX + GST per sample and diagnostic testing (i.e. non-scheme) is \$XXX + GST. NZVP is happy to receive and refer samples on for *B. ovis* testing. However, please be aware that Gribbles charge NZVP a minimum case fee of \$XXX + GST for testing referred to Gribbles. Therefore, if you are sending in 1 or 2 samples for scheme *B. ovis* CFT testing, unfortunately the charge for the case will be \$XXX + GST due to the minimum case fee we are charged. In such cases, you may wish to send the sample directly to Gribbles or alternatively send in at least 3 samples to ensure no minimum case fee is applied. If you have any enquiries about the *B. ovis* testing scheme, please do not hesitate to call the lab.



OESTRONE SULPHATE

The Oestrone Sulphate test is useful for determining pregnancy in mares >100 days post-mating. Until recently, NZVP was performing this test at both laboratories with a same day turnaround time. Unfortunately, the manufacturers of the test kit have postponed supply of the assay and it is unknown when the test will be available again for use in our labs. The serum quantitative Oestrone Sulphate pregnancy test is available for the same cost (\$XXX + GST) but the turnaround time is now 3-4 days (instead of same day). There is also a new Oestrone Sulphate test kit available for pregnancy testing on fresh urine. The urine must be tested within a few hours of taking the sample and it is ideal for testing on site at the farm or stud. If you are interested in finding out more information or purchasing this product, please contact Keith Henderson or Kim Wearne at AgResearch, Hopkirk Institute, Palmerston North (Phone: 06 351 8696)

BVD AND EBL PCR

Genemark (LIC) performs a combined BVDv/EBL PCR test for \$XXX + GST per sample for the first 30 samples and \$XXX + GST for subsequent samples in the same case.

The test is done only on 10ml EDTA/Purple Top Tube and turn around time ranges from 2-4 weeks.

Please keep the turnaround time in mind when requesting this test, especially if animals are being screened prior to sale or transportation. The BVD Antigen ELISA and EBL ELISA tests are still available for obtaining results within 2-3 days. Please contact the Serology Department if you require any further information about these tests.

SYNAPSE SURVEY

Thank you to everyone that completed the Synapse survey included in the last newsletter. We have received some excellent feedback and good ideas for articles you would like to see in future issues.

Watch this space!!



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

OXACILLIN RESISTANCE IN STREPTOCOCCUS UBERIS

Over the past season, NZVP has received a few enquiries about the presence of oxacillin resistance in *Streptococcus uberis*. In one of these cases, several cows came up with a mastitis due to apparently oxacillin resistant *Streptococcus uberis* after cloxacillin dry cow therapy.

Oxacillin is recommended by WHO as the 'type' antibiotic for the class of semisynthetic beta-lactam antibiotics which are active against beta-lactamase producing *Staphylococcus aureus*. (Watts et al, 1995). This class includes the very similar agent cloxacillin, which is a popular dry-cow product. The main use of cloxacillin is against *Staphylococcus aureus* but it also (generally) has good activity against other gram positive cocci. Oxacillin is selected as the type antibiotic because resistance to oxacillin is the best way to detect methicillin resistant *Staphylococcus aureus* (MRSA), a major problem in the human field. MRSA is also sporadically observed as a cause of mastitis in New Zealand.

At NZVP, reporting of resistance to a particular antibiotic is based on measurement of the diameter of a zone of clearance surrounding antibiotic-impregnated discs. With the exception of a few drugs used exclusively in the veterinary world, all of the standard 'zone diameters' established to measure resistance are based on human drugs and human pathogens. Individual zone diameters for most antibiotics have not been established for most mastitis pathogens (with the exception of agents like *Staphylococcus aureus*, which also commonly causes disease in humans) and commonly used drugs.

The issue of oxacillin resistance in *Strep uberis* has arisen recently, but going back in to the database we can see that it has been around for a while. Since NZVP's inception, 1345 isolates of *Strep uberis* have been tested against oxacillin. Of these, 234 (17.4%) are resistant. 57 (24.4%) of oxacillin resistant isolates are also resistant to penicillin. The remainder of oxacillin resistant *Strep uberis* (75.6%) remain sensitive to penicillin.

The resistance of *Strep uberis* to oxacillin/cloxacillin is reported in the literature, although not widely. In the main, studies evaluating cloxacillin as a dry cow therapy have focussed on *Staph aureus*, which is this drug's intended target. However, a survey has been done in the US (Watts et al, 1995) which identified *Strep uberis* isolates that have elevated cloxacillin minimal inhibitory concentrations, suggested by the authors to be due to strain dependent variation. Another study determined that 3.8% of *Strep uberis* isolates were oxacillin resistant (Rossitto et al, 2002). Interestingly, a similar study on cows in New Zealand and Denmark did not detect oxacillin resistant strains of *Strep uberis* (Salmon et al 1997). These studies were all based on determination of minimal inhibitory concentrations, not on disc diffusion assays, and likely were a more precise estimate of what happens in vivo.

The mechanism of oxacillin resistance is also a matter of debate. Most microbiologists explain organisms that

demonstrate oxacillin and penicillin resistance as having enhanced beta-lactamase activity. However, a significant proportion of isolates that we deal with here at NZVP have oxacillin resistance but seem to remain SENSITIVE to penicillin. This type of phenomena has been observed in human field in association with *Streptococcus pneumoniae*. A study of this pathogen showed that this was due to mutation in a gene encoding penicillin binding protein, which under enough selective pressure (i.e. the continued presence of oxacillin/cloxacillin), could result in the production of strains of the bacterium that were sensitive to penicillin but moderately resistant to cloxacillin (Dowson et al, 1994).

So what does this mean to you in making decisions about dry cow therapy? A few points to keep in mind:

- 1) The disc diffusion assay used as the standard in detecting antibiotic resistance is a relatively crude test at best. The test may not accurately reflect the levels of antibiotic which can be achieved in the udder.
- 2) The level of resistance to oxacillin in *Strep uberis* is probably overestimated using the disc diffusion assays, as surveys done recently in NZ and overseas using MIC determination show that resistance is much lower.
- 3) Decisions regarding dry cow therapy should be based on your knowledge of the pathogens causing most of the problems in a herd. Especially for many herds which have a high prevalence of *Staph aureus* mastitis, cloxacillin based preparations likely still remain a good choice.
- 4) A low prevalence of oxacillin/cloxacillin resistant *Strep uberis* may exist in New Zealand. Bacteria continue to evolve, and it is possible that the situation will change given enough selection pressure. We cannot assume that a particular antibiotic will remain effective indefinitely.

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References:

Salmon, S.A. et al. Minimum inhibitory concentrations for selected antimicrobial agents against organisms isolated from the mammary glands of dairy heifers in New Zealand and Denmark. *Journal of Dairy Science* 81:570-578, 1998.

Watts, S.A. et al. Antimicrobial susceptibility of microorganisms isolated from the mammary glands of dairy heifers. *Journal of Dairy Science* 78: 1637-1648, 1995.

Rossitto, P.V. et al. Antibiotic susceptibility patterns for environmental streptococci isolated from bovine mastitis in central California dairies. *Journal of Dairy Science* 85: 132-138, 2002.

Dowson, C.G. et al. Genetics of oxacillin resistance in clinical isolates of *Streptococcus pneumoniae* that are oxacillin resistant and penicillin susceptible. *Antimicrobial Agents and Chemotherapy*, 38(1): 49-53, 1994.

DIAGNOSING CALF DIARRHOEA

It can be a depressing experience to arrive on a farm with a group of sick calves and although we have passed the peak of the newborn season, there will be ongoing problems. Here are a few tips to help you survive.

History/clinical signs

Age – Age determines susceptibility to some diseases. An accurate age also helps you to interpret GGT results since these values decrease with age.

Colostrum intake – Take some serum (red top tube) to measure GGT levels, which give an estimation of colostrum intake in animals under 15 days old.

Clinical signs – the basics – temperature, respiration, pulse, demeanour, presence of scour (or not), presence of nervous signs (or not). Even if scour has not been observed by the farmer, neonatal calf diarrhoea should still be considered as a possibility, especially if the calf is dehydrated.

DIFFERENTIAL DIAGNOSES FOR CALF DIARRHOEA

Pathogen	Age	Lab Tests Available	Calf Panel	Comments
Enterotoxigenic E. coli (K99)	3 days or less	Antigen test on faeces Histology on rapidly fixed intestinal specimens*	<1 week	Calves over three days old are generally not susceptible
Attaching and effacing E. coli	20 - 30 days	Histology on rapidly fixed small intestinal specimens*	not included	Very rarely identified on histology
Rotavirus	5 - 15 days	Antigen test on faeces	<1 week panel 1-3 week panel	Very commonly identified
Coronavirus	5 - 21 days, may affect older animals	Antigen test on faeces	>3 week panel	Uncommon. Test younger animals if Rota negative.
Cryptosporidium	5 - 35 days	Stain on faeces Histology on rapidly fixed small intestinal specimens*	<1 week panel 1-3 week panel	Very commonly present in conjunction with Rotavirus.
Salmonella	0 - adult	Culture of faeces Histology on a variety of tissues, including large and small intestine*	All calf diarrhoea panels.	Quite common, causes deaths and sepsis. Outbreaks can result in heavy losses.
Coccidiosis	30 days and older	FEC/coccidia Histology on intestinal sections*	>3 week panel	Only in older calves and adults.
Campylobacter jejuni	possibly older animals	Culture	Not included	Questionable significance in the bovine. Can occur in normal animals.
Yersinia pseudotuberculosis and enteritidis	generally older than 3-4 months	Culture Histology on intestinal specimens*	Not included.	Common in animals older than 3-4 months.
Bovine Virus Diarrhoea	0 - adult	BVD antigen testing or PCR on EDTA sample* (can use red top tube for antigen testing over 3 months old)	Not included.	Persistently infected animals may become evident at any age.
Gastrointestinal Parasitism	approx 3 months - adult	FEC Serum pepsinogens for Ostertagiasis*	FEC included in >3 week panel	Very commonly identified in older calves and adults.
Johne's disease (Mycobacterium paratuberculosis)	Adults 2 years of age and over	ELISA on serum* Examination of faeces for acid-fast bacilli (not very sensitive)*	Not included.	Common cause of diarrhoea and wasting in adult animals only.

(ref: Radostits, O.M. et al. Veterinary Medicine, 9th Ed. W.B. Saunders, Toronto, Pages 780-781, 2003.)

* Please note that histology and blood tests are never included in a calf diarrhoea panel – only testing done on the faeces is included in these panels.

What if I have a dead calf (that has been dead for over 20 minutes)?

- 1) Take a sample of fresh lung when you first open the carcass – it's a good place to culture if sepsis is a possibility.
- 2) Next move on to the GI tract. Take a sample of faeces or large intestinal content for a calf panel, faecal egg count, or cultures. About half a pottle gives ample for all tests, but any faeces is better than none! If the intestinal tract is completely empty submit pieces of fresh large and small intestine instead. If there are very little faeces in the GI tract than enteric disease is even more likely.
- 3) Take histology samples from large intestine, small intestine, lung, liver, kidney, abomasum, myocardium. Place in formalin promptly and open out intestine samples so the formalin comes in contact with the mucosa.

- 4) Scout around the carcass for other evidence of infection – check the umbilicus for thickening, check a few joints for redness/fibrin, feel the lungs for evidence of respiratory disease.
- 5) Consider taking the brain out and submitting for histology if there is a history of nervous signs. Septic meningitis is quite common in colostrum deprived calves.

What if I have a moribund calf to euthanize or one that has been dead less than 20 minutes?

Do the above, but since the gut histology will be of more value, focus on taking these sections first and getting them in to formalin quickly. However, don't neglect to send in a sample of faecal content! Histology and fresh samples from the rest of the carcass (as above) may also be useful.

What is the most useful/cost effective testing?

The calf diarrhoea panels have been designed to give you the most useful and cost effective tests grouped together. However, you may wish to select your own tests individually. If you do this, and the tests make up a panel which is cheaper than the individual tests, we will always give you the panel price.

It may well be useful to do a necropsy as described above and submit the fixed and fresh tissues to NZVP for us to hold. That way, if the routine calf diarrhoea panel is unrevealing we can go ahead with histology.

If groups of animals are affected it may be worth testing several animals from a group to get an idea of what is happening in the herd. No test is 100% sensitive. In Rotaviral infections, for instance, viral antigen tends only to be shed in the initial stages of the diarrhoea. Later on animals will test negative even when they have severe diarrhoea due to Rotavirus. Salmonella may be intermittently shed. If you are concerned about cost, you can submit several samples of faeces but only have us test one or two (with the rest on hold). That way, we have the extra samples on hand if testing the initial samples is not revealing.

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BVD ANTIBODY BULK TANK MILK TEST

The manufacturers of the BVD Antibody Bulk Tank Milk (BTM) ELISA test recently informed us of a change in the production of the kits which resulted in reduction in sensitivity of the assay. Whilst the manufacturers run quality control testing on their products, the effect of the change has only become apparent over the past few months upon retrospective trend analysis of results. The reduction in sensitivity of the test means that herd prevalence results reported for the BVD Ab BTM test were likely to be lower than the actual level of BVD Ab prevalence in the herd (e.g. a reported result of 10-30% prevalence may actually have a prevalence level > 30%). Due to most veterinary laboratories in NZ using the same test kit, it has affected many veterinarians and farmers. NZVP has already notified the affected clients with a letter and testing vouchers.



Currently, the BVD Ab BTM ELISA test is unavailable. This event has prompted an industry wide validation of BVD Antibody Bulk Tank Milk ELISA tests available on the market. The outcome of this trial is expected in late October/early November with resumption of testing after this time. In the meantime, if you are interested in testing a milk sample for the presence of BVDv infection, the sample can be sent for BVD Ag PCR test which is currently available. If you have any questions, please do not hesitate to contact the Lab.

TRACE ELEMENT UPDATE: BOVINE SERUM COPPER REFERENCE RANGE

In response to the findings of a recent study bovine serum copper reference ranges have been modified slightly. The adequate range for serum copper was previously 8 umol/L – 25 umol/L. It is now 8 umol/L – 20 umol/L. This change provides a better correlation between serum copper and ferroxidase whilst still identifying those animals that may have elevated results as a consequence of underlying inflammation.

Reference:

Laven RA, Lawrence KE, Livesey CT. The assessment of blood copper status in cattle: A comparison of measurements of caeruloplasmin and elemental copper in serum and plasma. *New Zealand Veterinary Journal* 55(4), 171-176, 2007.