Paws claws and udder things

Gribbles VETERINARY

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Larva palaver

LAWRENCE MCMURTRY

We often get asked why we don't recommend refrigerating (or even chilling) faeces requiring a culture for third-stage nematode larvae or when looking for stage-one lungworm larvae.

Cold storage of faeces can affect the development and composition of parasites in larval cultures and extraction of lungworm.

Third-stage larval cultures

Ovine nematode species are affected to varying degrees after refrigeration at 4°C. The time spent chilled also impacts the subsequent larval culture results (McKenna, 1998).

McKenna reports that ovine *Ostertagia* (*Telodorsagia*) and *Trichostrongylus* species are the least affected after 24 hours refrigeration but after three days refrigeration, *Oesophagostomum* and *Chabertia* rapidly decline in development.

Cooperia and *Haemonchus* are the most affected. Refrigeration for 24 hours can reduce egg viability of these species by approximately 30% and 25% respectively; and after seven days at 4°C, only 10% of *Haemonchus* eggs were found to be viable (McKenna, 1998).

Samples refrigerated overnight and then sent on ice to the laboratory will result in inaccurate assessments of larval culture composition.

Egg and larval development

Most Nematode eggs do not begin developing until the ground temperature rises above 10°C. An exception is *Telodorsagia* which can start slowly developing from 4°C (Vlassoff and Charleston, 1982). Conversely, ground temperatures greater than 30°C together with dry conditions can desiccate first-stage nematode larvae. A small proportion may resume development once they receive moisture from rainfall. Under field conditions, the rate of development to third-stage larvae can take two to three weeks or longer.

Once nematodes have developed to third-stage larvae they are however a lot more resistant to cold and desiccation. This is because they retain their protective sheath and are in a hypobiotic state: non-feeding, have stored energy cells, and are waiting to be ingested by a potential host.

Nematodirus is an exception. These nematodes store all of the energy for development in the egg, so develop and hatch as third-stage infective larvae.

In ideal laboratory conditions - a good balance of moisture but not too wet, enough oxygen by mixing with vermiculite to help aeration and a temperature between 24-28°C - up to approximately 90% of larvae can be recovered. Under these conditions eggs can hatch (most within 24-37 hours) and develop to third-stage larvae in 5-7 days.

Nematodirus are again an exception, as they take longer to hatch. For example, *N. spathiger* takes 10-15 days and *N.filicollis* can take 54 days. Since the large eggs of this genera are so easily recognisable, there is little reason for larval culture.

Lungworm larvae

The first-stage lungworm larvae rely on the warmth of the faeces to migrate and feed. The second- and third-stages do not feed but remain in the first-stage cuticle. The laboratory extraction process relies on active larvae, that have feed and the energy to pass out of the faeces and through a tissue. Therefore, being placed in a cold environment would prevent feeding and development of these larvae.

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Figure 1. Chabertia ovina third-stage larvae. Photo credit: Van Wyk, JA. J Vet Res 44:197-199, 1977.

Recommendations

If you are requiring larval cultures or lungworm testing and there is a delay in transporting faecal samples to the laboratory (especially in summer), we recommend you split the samples. Place a portion of the sample for faecal egg count in the fridge (to prevent the eggs hatching) and leave a portion at room temperature for larval culture/ lungworm. Alternatively a wine fridge running at 10-15°C would both slow down egg development without inhibiting future development of third-stage larvae.

References

McKenna, PB., The effect of previous cold storage on the subsequent recovery of infective third-stage nematode larvae from sheep faeces. *Veterinary Parasitology* 80:167 -172, 1998.

Vlassoff A, and Charleston WAG. In *Control of Internal Parasites in Sheep*. (ed. Ross, AD.) Animal Industries Workshop, Lincoln College, New Zealand, 1982.

A slide worth a thousand numbers

LISA HULME-MOIR

This recent case highlights the importance of blood film examinations and illustrates how diagnoses may be missed if relying on in-clinic analyser counts alone.

Clinical history:

A blood sample from a 9-year-old male Labrador cross was submitted for blood film examination after presenting lethargic, off his food and with a temperature of 40.5°C. His popliteal lymph nodes were slightly enlarged but no other abnormalities were noted on physical examination. In-clinic diagnostics had found him to be anaemic with an apparent mild neutrophilia and mild lymphocytosis (Table 1). His biochemistry results were unremarkable.

Laboratory findings:

On examination of the blood film, the dog had a lymphocytosis with increased numbers of large atypical mononuclear cells most likely of lymphoid in origin (Figure 1). Instead of a neutrophilia being present, the neutrophil count was low with a degenerative left shift and toxic change. The anaemia appeared non-regenerative.

Put together, these findings were highly suspicious for lymphoid neoplasia (lymphoid leukaemia or leukemic stage of lymphoma). The neutropenia may have reflected secondary infection due to immunosuppression or a combination of effects from the neoplastic process (myelopthisis, tissue necrosis etc.).

Discussion:

This case demonstrates how analyser counts can sometimes be misleading. It is likely that the in-clinic analyser misclassified the neoplastic cells as neutrophils, thereby masking the presence of a neutropenia. Haematology analysers can produce very accurate differential cell counts in healthy animals, but are frequently inaccurate when cells with altered morphology are present e.g. band neutrophils, neoplastic cells, nucleated red cells.

Where possible, a blood film should be examined whenever haematology is performed on a sick animal and any time abnormalities are detected on in-clinic analyser results, whether the animal is sick or well. If a smear examination is not performed, keep in mind that you may be missing something important. For more information on blood film examinations see Kathryn Jenkins article in the June 2022 issue of VetScript.

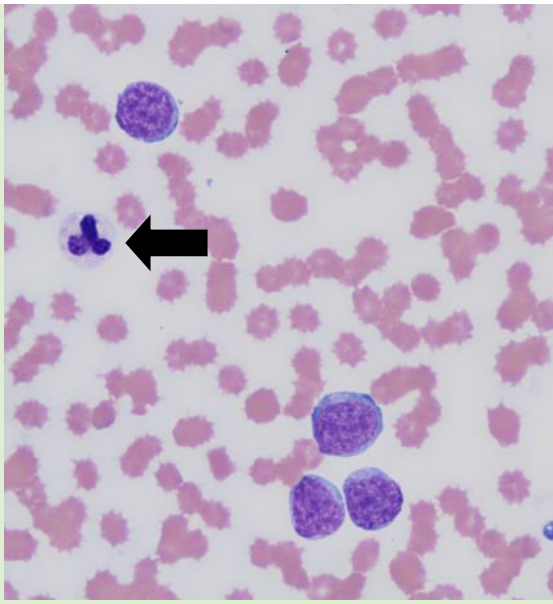


Figure 1. Photomicrograph of blood film from a pyrexic Labrador. Note the large size of the atypical cells compared to the band neutrophil (arrow).

Don't rely on analyser counts alone. Always review blood smears from sick animals and all cases with abnormal results.

Table 1. In-clinic analyser results from a pyrexic Labrador and subsequent results from an analyser leukocyte count (WBC) and manual differential cell count performed at the local laboratory. N/P = not performed.

	In-house analyser	Reference laboratory	Reference interval	
RBC	4.08 L	N/P	5.5 – 8.5	$x10^{12}$/L
HGB	8.4 L	N/P	12 – 18	g/dL
HCT	26.87 L	N/P	37 – 55	%
MCV	66	N/P	60 – 77	fL
MCHC	19.8	N/P	30.9 – 39.0	g/dL
WBC	19.92 H	20.7 H	6.0 – 15.0	$x10^9$/L
Neutrophils	13.74 H	0.4 L	3.6 – 11.5	$x10^9$/L
Band neutrophils	-	0.8 H	0 – 0.5	$x10^9$/L
Lymphocytes	6.0 H	9.7 H	1.0 – 4.8	$x10^9$/L
Monocytes	0.12	1.4	0.2 – 1.5	$x10^9$/L
Eosinophils	0.06	-	0.0 – 0.8	$x10^9$/L
Other cell line	-	8.3		

Thank you to Anna Tiernan, Vet Farm & Pet Clinic, Warkworth, for this interesting case and excellent fresh blood films.

Scalding rain

SANDY WELTAN

Clinical history:

Three smears were received from skin scrapings from cattle with diffuse skin lesions. The lesions were widely distributed but were particularly severe on the white areas of the skin (Figure 1).

Figure 1. Cattle beast with diffuse skin lesions.

Laboratory findings:

On cytology, the cellularity was high in all the smears and consisted of keratinocytes in a background of erythrocytes. Moderate numbers of inflammatory cells were seen including non-degenerate and degenerate neutrophils, lymphocytes and fibroblasts. Large numbers of bacteria with a "railway track" morphology were seen (Figure 2).

Laboratory diagnosis:

The distinctive bacterial morphology indicates infection with *Dermatophilus congolensis* - commonly referred to as rain scald.

Discussion:

Dermatophilosis has a worldwide distribution but is most prevalent in tropical and subtropical regions, associated with moist, humid conditions. It affects a wide range of domestic and a wild animals. In New Zealand, most cases are in cattle, sheep and cervids, but it has been described in cats and in humans.

Members of the genus *Dermatophilus* are aerobic, gram-positive, branching, filamentous rods. They produce motile zoospores, and aerial mycelia are ordinarily absent. Septa are formed in transverse and horizontal planes and give rise to parallel rows of coccoid cells, often referred to as “railroad tracks.” These organisms are catalase positive and non–acid fast. *Dermatophilus* is an obligate parasite so contact between animals is a prerequisite for infection. However, the organism can sporulate and the spores are resistant to desiccation and cause new infections when moist conditions return.

Dermatophilosis is characterised by the development of an exudative dermatitis followed by scab formation, alopecia and thickening of the skin. The serous exudate leads to the development of scabs that finally become hard, horny and confluent and they may have an almost wart-like appearance.

The two most important predisposing factors are skin damage and moisture. There was a history of facial eczema on this farm and the predominance of lesions on the white patches suggests that this may have been the initiating factor combined with the wet weather.

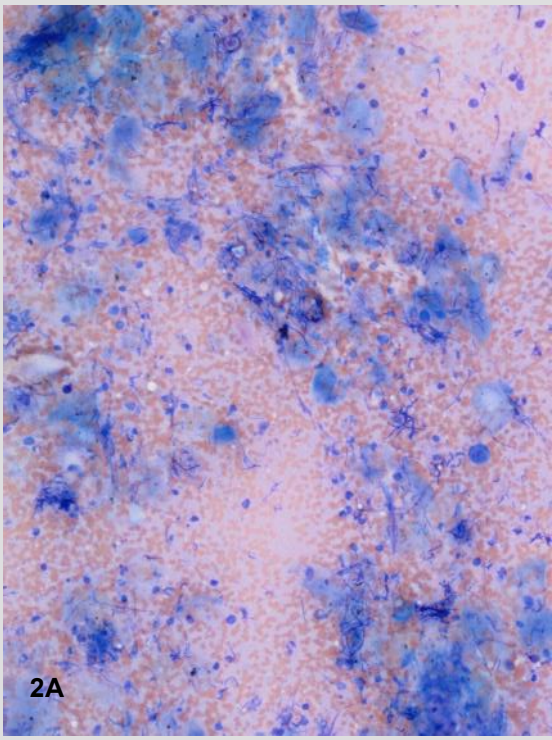
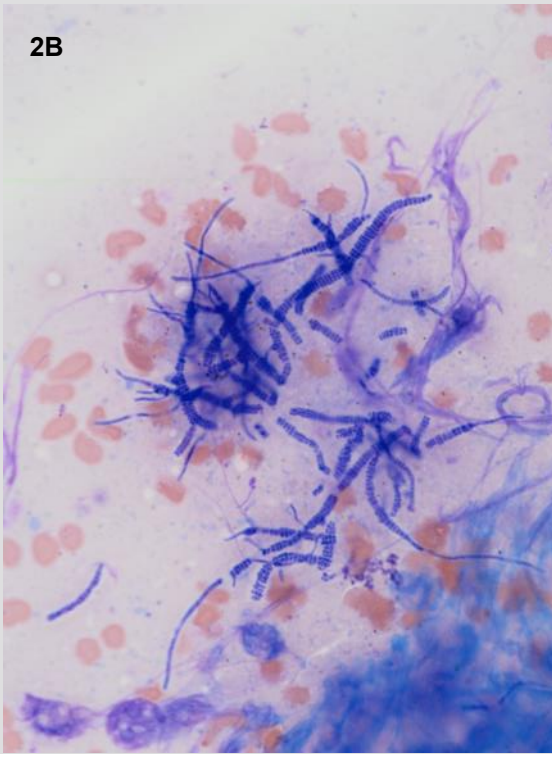


Figure 2A. Low power photomicrograph of smear—background of RBCs with inflammatory cells, keratinocytes and bacterial elements.

Figure 2B. High power magnification clearly demonstrating the classic “railway track” morphology of Dermatophilus congolensis.



Lab tip

If you are submitting a mixed bag of samples, please label the individual containers with the sample type so it is clear to us which sample is which. We sometimes have to rely on the less than scientific sniff test to distinguish formalin-fixed tissue from those in saline; the shake test to determine which is abdominal fluid and which urine; or we may even have to run a creatinine to find out which is the separated serum and which is the urine. So please don’t make us do the taste test on the possible faeces in the hummus container you sent in with some morning tea treats - help us out and clearly label them all!

Testing young calves for BVD

Calf screening in dairy herds aims to identify and remove persistently infected (PI) animals before they can infect any pregnant cows.

An ideal time to sample calves is during tagging or disbudding. The antigen ELISA is a suitable test to use on ear notch samples for animals in this age group. PCR is a suitable test for serum or ear notch regardless of age.

PCR vs antigen ELISA – which to choose?

Tissue samples are tested in a pool by PCR, then all samples in a positive pool are individually tested by antigen ELISA.

Serum antigen ELISA is still NOT a suitable test for animals less than 35 days old due to the potential for false negative results. When serum samples are tested using PCR, the sera are pooled for PCR testing and then all samples in a positive pool are individually tested using PCR.

Benefits of PCR testing:

PCR testing is highly sensitive and will detect transiently infected animals (TI) as well as PI animals. For young calves, we recommend testing tissue samples by PCR. We use a combination of PCR (pooled samples) then antigen ELISA (on individuals in positive pools), which allows for differentiation of TI and PI animals in most cases.

Note: As there are no clear cut-off value between TI and PI animals, Gribbles Veterinary always recommends retesting positive animals 4-weeks later to minimise the chances of culling a TI animal, regardless of whether the positive test is completed by PCR or antigen ELISA.

Summary of testing options:

Animal age	<35 days	>35 days
PCR	Serum or ear notch	Serum or ear notch
Antigen ELISA	Ear notch only	Serum or ear notch
Result type	Individual	Individual

In brief

- **Due to domestic supplier issues,** we now must source our D-formalizer offshore. Consequently the price has increased to $45.33 (ex. GST), effective 1 September 2022. This product is a must-have in your clinic if you wish to dispose of formalin—it neutralises formalin and enables it to be safely disposed of down the drain. Find it online here.
- **Thank you** to everyone who participated in our client satisfaction survey. We are busy collating the results and will be able to share findings with you in the next newsletter.
- **Histology testing** is now back on track! Thank you to everyone who sent in kind words of support for our team, it was much appreciated.

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