Science, Worms, and Poo

By Stephen Mulholland, Ph.D.

I was asked to write up the talk I gave at the AGM in May 2009. This is my attempt to do so. In written form you will miss my tangential interludes, wild gesticulation, and occasional sound-effects. You will have to ask the people who attended the AGM if that is better or not.

Part 1: Science and Llamas

People who buy llamas tend to be very concerned with the health and welfare of their furry companions. Books, magazines, the internet - all are trawled, looking for hints and tips. But it is beneficial to remember an old joke here, "Ask four llama breeders a question and you are likely to get five answers!"

Compared to most other stock species we still know very little about camelids. Medicines are used off label, Veterinarians do their best to apply knowledge from other ruminants, and we all collect stories and anecdotes about things that work, and what went horribly wrong. This accumulated wisdom is the basis for much of what we now consider "best practice."

But how can you know what to trust? Which of those "five answers" is the right one? Are any of them the right answer? Here I think it is very useful to apply some of the rigorous intellectual techniques that we call "the scientific method." This is a means of asking questions such that the results you obtain are very likely to be correct. There are five key features to this method: Observation; Description where the information must be reliable, replicable and valid; Prediction, the information must be valid for the past, present, and future; Control, meaning you must fairly sample the range of possible occurrences; and Falsifiability, the elimination of plausible alternatives while the theory remains open to disproof.

I will not get into an in-depth look on how the scientific method is applied, there are large books available on the subject. The question is, how do I know what sources of information I can trust?

Best - are published in a peer-reviewed journal. These require the methods used by the author to be stated, and the data and analysis to stand up to review by independent experts in the field.
Good - is written information that is done in a "scientific" manner. The author publishes the methods used to collect the information, the data is presented, and then the conclusions are drawn. This format allows you to see how the author reached their conclusions, and decide whether you agree or not.
The Rest - simply makes a statement, with no empirical evidence to back it up. They might be right, they might be wrong. Without data and attributed sources it is difficult to separate the wheat from the chaff.

The key is critical thinking. Don't automatically trust what someone says just because they have 10 or 20 years of experience, or they have a string of letters after their name. Don't ignore them, simply remember that what they say may or may not be correct. Even a fact that has been "proven" might later be disproven, this is the essence of falsifiability, no matter how hard we try, even the best experiment can get it wrong.

Remember: Something I have told you, or will tell you, is wrong! But I don't know what it is! The goal is to always be expanding our knowledge and understanding, keeping our minds open, and being willing to freely admit when we have gotten something wrong.

Part 2: Worms in your Llamas

Llamas play host to at least 16 different species of major parasites (1), though some of these are thankfully not found in New Zealand. What I am going to discuss here are the internal parasites, the worms that live in the gut, muscles, and internal organs. Parasites of the abomasum (compartment 3 of the stomach), small intestine, and colon are the main problems we face daily. Thankfully these parasites share a feature - their reproductive strategy involves ejecting their eggs out the backside of the llama, where they hatch on the pasture and infect other llamas. These faecal eggs can be easily measured, and provide a great tool for detecting which llamas have a problem.

Parasites follow a life cycle where adult worms in the llama produce thousands of eggs. These are excreted in the poo and fall onto the pasture where they hatch into larvae. These larvae go through multiple life cycle stages, until they are consumed by a passing llama and can start the cycle anew. Most (~95%) of the parasites on your farm exist as larvae on the pasture. Larvae can only migrate about 30cm from their original poo, so the

concentrations of larvae will be highest right around the midden. When you push your llamas so that they start grazing on or near the middens it raises their risk of ingesting too many parasites.

Most internal parasites are quite host specific, if the "wrong" species consumes the larvae, it will die. This provides a very useful pasture management tool, by "cross-grazing" with a non-compatible species, you can kill large number of larvae. Horses and cattle work well for this, doubly so because they will immediately target the long, lush grass around the midden. Sheep and goats are not a good choice, they share many parasites with llamas, and will just help to spread the worms away from the midden and all over the paddock.

Parasites have environmental preferences - you will find a different mix in Northland than in central Otago. I have been told by an Otago vet that she sees more Nematodirus in her area. Here on our farm in the Wellington area I see primarily Cooperia / Osterlagia / Trichstrongylus eggs (I don't know which, the eggs look nearly identical). I know up north there is a much greater risk of haemonchus contortus, the dreaded barber's pole worm. It is important to remember that as llamas travel around the country, so do their parasites. While it is true that haemonchus contortus cannot over-winter from Canterbury southwards, they can do quite well from Spring through into Autumn. It is possible for these quick-breeding parasites to go through multiple generations in that time. Don't be complacent! With animal movements and a changing climate, old assumptions about what is a threat where may no longer hold true.

Of course the most pressing question is, "When do I have a problem?" Llamas are notoriously stoic, and just looking out your window at the herd is unlikely to help you spot problems early. Here are some commonly used techniques:

- Scouring. This is a real mixed bag. Heavy worm burdens can cause scouring. But so can a 24-48 hour "tummy bug". We have a girl who can have 850 eggs/gram in her poo (a really high level!), and still be dropping perfect pellets. Another started scouring but never had eggs that I could count, it turned out that she had cancer! So scouring is not the greatest indicator.
- Weight loss/ill thrift. This can be a much better indicator. Most worms cause a constant drain which will result in the animal being thinner. This might not mean "thin" (BCS 1 or 2), merely thinner than its companions. If you had a mob of geldings who were all BCS 5, and during your monthly check suddenly one has dropped to BCS 3 it would be worth checking for parasites.
- Sudden Death. This is an indication of a problem, but a bit late to do much about it. This can occur in severe cases of *haemonchus contortus*.

I find the Fecal Egg Count (FEC) to be a great tool, and you can do it yourself at home (more on that below). We have more than 50 camelids under our care, and I don't care to spend all my time counting eggs, so I use the FEC as a diagnostic tool to determine if an animal is having problems.

The easiest way is to look for the animal that stands out. Look at groups of animals that are under the same grazing and metabolic conditions (all geldings, or all females with cria at foot, or all yearling tui). Does one or two of them stand out by being thinner, lagging, or looking unhappy? If so, one good health check is to count the eggs in their poo!

There are limits to this, however. Only adult worms produce eggs, and it is possible for juvenile worms to make your llama very sick. There are also some circumstances where adult worms can stop making eggs. Some species of parasites generate very few eggs, so a "low count" might come from an animal with lots of worms. Due to this variability it would be good to talk to your vet about what worms are common in your area, and learn about what you should be looking for. If you are doing your own counts it would probably be very helpful to split your initial samples - you do your own count on half, and send the other half to your vet for professional lab counting (This will be $5-20 per sample, depending on the vet/lab). This way you can ensure you are doing it correctly (you get the same count as the lab), and you will have the eggs professionally identified, and the vet can inform you as to the risks and proper treatment for those parasite species.

The other question is simply: how many eggs are too many? Seeing a few eggs (50 to 150 eggs per gram) in an otherwise fat and happy llama is no cause for panic. It is natural and normal for all llamas to have some parasites. As a general rule, if I see any eggs in a thin animal, I will treat it with drench/wormer. If the parasites were the cause, the animal generally starts looking better within a week or two. (That being said we had one girl last winter we had to treat 4 times for worms, she got "behind the curve" and had a tough time during the cold weather which allowed the larvae to keep re-infesting her. Now that she is a healthy BCS 4 she is doing much better at staying worm free, and we have not

had to drench her in more than 8 months.) If the animal is not looking better after drenching, then the worm infection might have been a secondary effect of whatever is making you animal sick/thin. (I must also confess that I don't always FEC before treating a thin animal with wormer/drench. The main girl-mob has about 45 animals, and it is very difficult to get them to "poo on command" so we can collect a fresh sample. While I could directly obtain a sample using a gloved, lubricated finger, that is a stress that neither I nor my alpacas/llamas want under normal circumstances.)

When it comes to effective parasite treatment, remember that prevention is always better than cure. Limit the challenges - any animal can be overwhelmed if it eats too many larvae. Pushing the llamas to eat down the middens raises the risk (though I know sometimes it cannot be avoided when feed gets short). Adult animals when well-fed and stress-free can develop strong immunity to parasites. Cultivate practices that help this natural
immunity (only drenching animals that need it, not over-stocking, providing adequate shelter from bad weather, arranging your herds to mitigate inter-animal strife and stress, etc.). Also, if it is practical, look into cross-grazing your paddocks with horses or cattle. If you have a friendly neighbour, this could be a good reciprocal arrangement, as your llamas will do a good job vacuuming-up and killing all the horse and cattle specific parasites.

But eventually one of your llamas will get wormy, and then it is time to pull out the drench (wormer). These chemicals, when used properly and sparingly, are a wonderful tool of animal health. Drench should only be given to animals when they need it, not blindly administered to the whole herd based on a calendar-based "to do list."

There are three families of drench available; Benzimidazoles (white drench), Levamisole (clear drench), and Macrocyclic Lactones (the "-ectins", e.g. Ivermectin). A fourth family was just recently premiered. As we don't yet know the safety margin for this chemical in llamas I recommend staying away from it for now. (It's chemistry and activity suggests that it will probably be safe and effective, I just don't want to be the person who first finds out just what the safety limit is.)

The really important part is to give enough! Under-drenching is one of the quickest ways to develop drench resistant worms. There are always some resistant worms in every population, our goal is to minimize the spread of resistance.

For the Benzimidazoles and Macrocyclic Lactones that means giving your llama 1.5x the sheep dose, based on the weight. Levamisole has a quite narrow safety margin, and I generally don't recommend it. You should not give more than the sheep-dose (based on weight again). I prefer injectable drenches, as I think it is less stressful for the llama. There is also the problem that llamas are quite good at spitting back up some unknown amount of oral drench, making it impossible to know just how much of a dose you delivered.

If you are using Dectomax, you should be administering 5 to 6 ml of drench to your average adult llama! (1 ml per 25 kg body weight) The Macrocyclic Lactones have a huge safety margin (up to about 10x the sheep dose), so your llama is in no danger from a 6 ml dose (it would take quite a slip-up to accidentally inject 45ml!).

After you drench your llama, you should put it back onto contaminated pasture. This may seem counter intuitive- why not put it on the "cleanest" pasture? Simple- after drenching the only worms remaining are the partially or completely drench-resistant ones. If you put the llama on a clean pasture, all of the eggs coming out in the poo will be resistant, and that pasture suddenly has a greatly increased population of resistant worms. When the llama is put back onto contaminated pasture, the resistant eggs get dropped among all the non-resistant ones, and the larvae that hatch can go on to breed with non-resistant mates, and hopefully dilute away the resistance genes.

Most of us drench, and simply hope it worked. But there is a simple test which measures drench effectiveness. Do a FEC before you drench, and again 10 days after you drench. You should see a 95%+ drop in worm numbers (which at the worm numbers we work with, means no eggs should be spotted 10 days post-drench). If you don't, you had a problem with your drench (either resistance in the worms, or the drench was not administered properly).

If the worms go away (based on FEC), but the animal remains thin and sick, then the worms were not the (primary) problem! In that case the animal may have been wormy because another condition weakened it and left it vulnerable to infection, it is time to look for other causes. Talk to your vet.

Part 3: Faecal Egg Counts

Fun with poo! And a very useful animal management tool which we can all do at home without fancy equipment.

Poo is full of "stuff", the digestive debris that is a mix of well-chewed grass, bacteria, and other digestive by-products. How are we going to spot the eggs amongst all that other stuff? There is a simple solution- floatation! If we mix the poo into a solution with an appropriate specific gravity, then the eggs will float, and everything else will sink to the bottom.

The so-called "floatation method" is what I use, and is generally quite effective. Some alpaca breeders up north have moved to a version of this which also uses a centrifuge, as they find it is better for picking out *haemonchus contortus* (Barbers Pole Worm) eggs. If you want more information on the centrifuge technique, please email me. (Stephen@suncrow.com)

You will need:

Fresh Poo

A scale (accurate to 0.1 grams- I purchased a small hand-held scale for about $135. It was the most expensive bit of my setup. I have been told though that there are some much lower prices available on TradeMe)

A salt or sugar saturated solution (easily done by adding either table salt (Sodium chloride, NaCl) or table sugar (Sucrose) to water until no more will dissolve. You should have about a cm of salt or sugar on the bottom when you are done. This solution will keep for some weeks in a cool, dark place.)

A way to measure 28 ml of solution (medicine measuring cups available at pharmacies work well enough)

A mixing vessel (mug, glass, beaker)

An eye-dropper

A counting slide (McMaster or other brands. They run about $50 to $75, but should last for years)

A microscope capable of 100x magnification (kids "educational" models available via TradeMe for about $50 to $100, nice new bifocal ones are also available if your budget allows)

The method:

Weigh out 2 grams of poo
Mash the poo up in 28 ml float solution
(Optional- drain through tea strainer to remove biggest "chunks")
While constantly stirring take liquid from middle with eye-dropper
Put mixed liquid into counting slide
Wait 2 minutes
Count both chambers (the boxed-area in the middle of the slide)

Total eggs counted x 50 = eggs per gram (epg).

With your microscope you want to focus on the top of the chamber, where the eggs have floated above all the other "stuff" in the mix. The easiest way to do this is to look for air bubbles. These are bright-centred circles with a very dark edge. Air bubbles vary in size, and depending on your technique, you may have a lot of them. Once you find and focus on an air bubble you know you are focused at the correct depth in the sample, and you can start scanning for eggs. Most cheap microscopes do not have a moveable stage, so you will have to slowly and carefully move the slide back and forth so you can scan the whole area for eggs.

I should mention accuracy here. The goal is to perform each step as accurately as possible. But this isn't always easy when using somewhat improvised equipment. Inaccuracies add up, but for tests such as this they aren't the end of the world. For example, if you are using a measure cup for your 28 ml of floatation solution that is not very accurate, such that you get somewhere between 26 & 30 ml of floatation solution, then your final results will have an additional +/-10%. So if you measure 3 eggs and then multiply 3 x 50 = 150 eggs per gram, then your results could be between 135 and 165 epg (i.e. 150 ± 10%). Likewise if your ability to weigh out the 2.0 grams of poo is not perfectly accurate, error will creep in there, too. (Issues of accuracy and precision can get quite complex in scientific studies. *(continued pg38)* For our purposes sticking to the method as closely as possible will give the best and most accurate results).

If the goal is to get a very accurate Faecal Egg Count Reduction Test so you can distinguish between 85% worm kill and 95% worm kill, then those inaccuracies will completely flummox your experiment. If your goal is simply to determine "does my llama have eggs in his poo?", then small (and even medium) sized errors are okay.

One good trick to check yourself (whether you are using accurate methods or not) is to collect a fresh sample and split it. Send half to a professional lab (usually through your vet), and test the other half yourself. See if the results compare.

Another good trick is to "over count". Instead of counting the 2 areas on the slide and multiplying by 50, count 4 sections and multiply by 25, or if you are very keen count 8 sections and multiply by 12.5. (This will require cleaning out your slide between counts, of course.) These over-counts can give you a much lower margin of error, and it the best way to measure worm egg levels animals with a very low count. (If the llama has less than 50 epg, then luck means that many times when you count you might get no eggs in either chamber, so the count reads as "zero" even though there are some eggs in the animal). *SM*

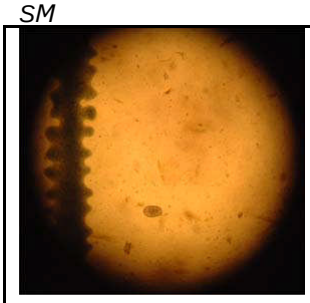	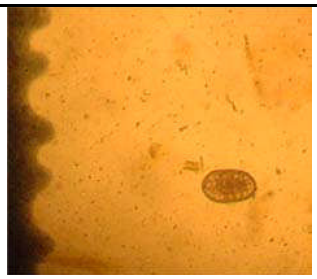
Image showing the field of view at 100X. Your microscope may have a slightly larger or smaller field of view. The oval structure is a *haemonchus contortus* egg. Below and left of the egg is a bit of plant-cell debris. The black stripe at left is a grid line on the counting slide.	The same egg at 200X magnification. *Cooperia, Haemonchus, Osteragia, Trichostrongylus, Camelostrongylus, and Oesophagostomum* eggs all look very similar and are all about 80 mm by 40 mm. (1mm = 1 micrometer, or 1/1000th of a millimeter)
	
Nematodirus egg at 400X. *Nematodirus* eggs are large (200 mm by 90 mm) making them very easy to spot. This parasite is a low egg producer, so even low epg counts could indicate problems.	Coccidia oocyst at 200X The organisms that cause coccidia are very small, and can be difficult for inexperienced uses to spot. (*Eimeria llama* 38 x 28 μm, *Eimeria alpaca* 26 x 21 μm) Coccidia also has only a short period during which large numbers of oocysts are produced, so it is probably better to talk to your veterinarian if you think that might be the problem.

(1) Veterinary Parasitology Reference Manual Fifth Edition, William J Foreyt, Blackwell Publishing, pp 115-120

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