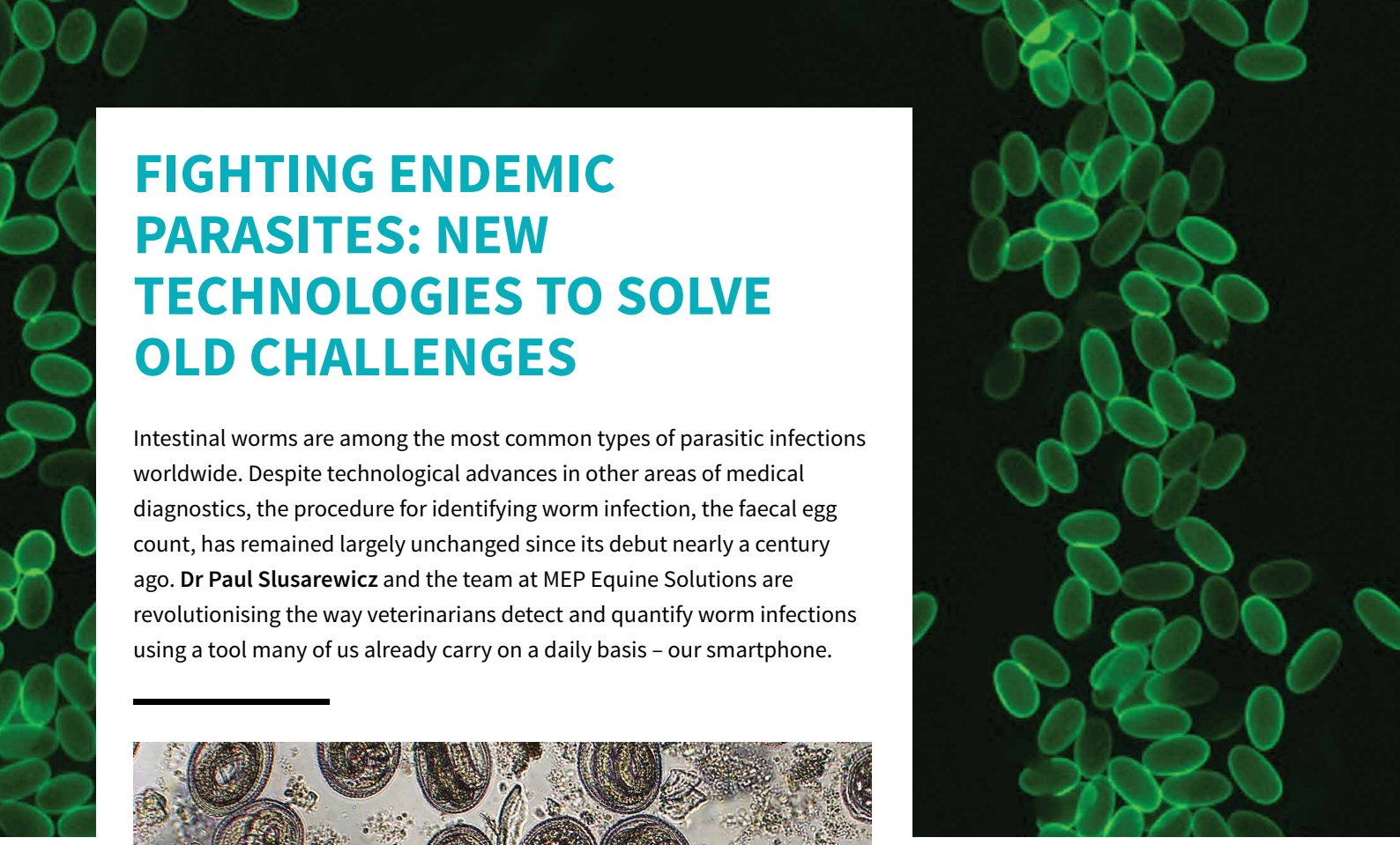
Fighting Endemic Parasites: New Technologies to Solve Old Challenges

Dr Paul Slusarewicz

Scientia



FIGHTING ENDEMIC PARASITES: NEW TECHNOLOGIES TO SOLVE OLD CHALLENGES

Intestinal worms are among the most common types of parasitic infections worldwide. Despite technological advances in other areas of medical diagnostics, the procedure for identifying worm infection, the faecal egg count, has remained largely unchanged since its debut nearly a century ago. **Dr Paul Slusarewicz** and the team at MEP Equine Solutions are revolutionising the way veterinarians detect and quantify worm infections using a tool many of us already carry on a daily basis – our smartphone.

A Worldwide Problem

Intestinal parasites are a major concern for human health in developing countries and veterinary medicine worldwide. Helminth worms, such as roundworms, are the most common type of intestinal parasite in mammals and pose a major health risk to infected host individuals. They feed off of the host and commonly cause symptoms such as poor nutrient absorption, anaemia, muscular weakness, impaired immune function, and in severe cases, intestinal blockage.

Preventing and treating intestinal worm infections is of paramount concern in many species, particularly in grazing animals such as horses. The life cycle of many intestinal parasites involves the deposition of eggs in manure in the hope of transmitting to a new host animal through accidental ingestion or direct contact with the skin. When one member of a herd of grazing animals is infected with parasitic worms, others easily pick up the infection by ingesting grass contaminated with eggs and larva.

Historically, the standard for diagnosing parasitic worm infection has been the 'faecal egg count' – by analysing stool samples under a microscope. While this procedure is fairly simple in theory (owners just collect faecal matter, and send it to a laboratory), it carries many limitations. First of all, microscopic visualisation of parasitic eggs typically cannot be done on site, it requires technical expertise and expensive microscopic equipment, and it is time consuming, often taking days to obtain results. The results are often inconsistent, as they rely upon the skill, judgement and level of fatigue of the person doing the counting. Also, two egg counts of the same sample are seldom the same, due to the variation inherent in the sampling procedure itself.

Because of these limitations, many horse owners choose to skip this test. Instead they give their animals deworming drugs prophylactically on regular schedules with no knowledge of the horse's infection status, even though only animals shedding high amounts of eggs need to be treated to keep levels of infection low in the herd. However, this strategy carries its own major limitation – since deworming treatments are given to all the animals and are not targeted to the highest shedders, many more parasites are exposed to these drugs that would be the case with targeted treatment. As a result, the most drug-resistant parasitic worms persist, and highly drug-resistant parasites are emerging at alarming rates.

Dr Slusarewicz and his partners recognised the need for a simple and fast stall-side method of determining parasitic worm infection in horses, empowering horse owners to offer targeted treatment options. Their company, MEP Equine Solutions, has developed a now patented procedure, which has the potential to transform how veterinarians and doctors approach the identification and treatment of parasitic worm infections.

Fresh Ideas in Diagnostics

Dr Slusarewicz began his career as a biochemist, working to solve common health problems at the molecular level. After spending the first half of his career studying human health, he and his CEO Eric Hauck realised that a technology they were working on together to repair tendon proteins could be readily translated to fill a need in equine medicine. The vision for improved parasite diagnosis began in

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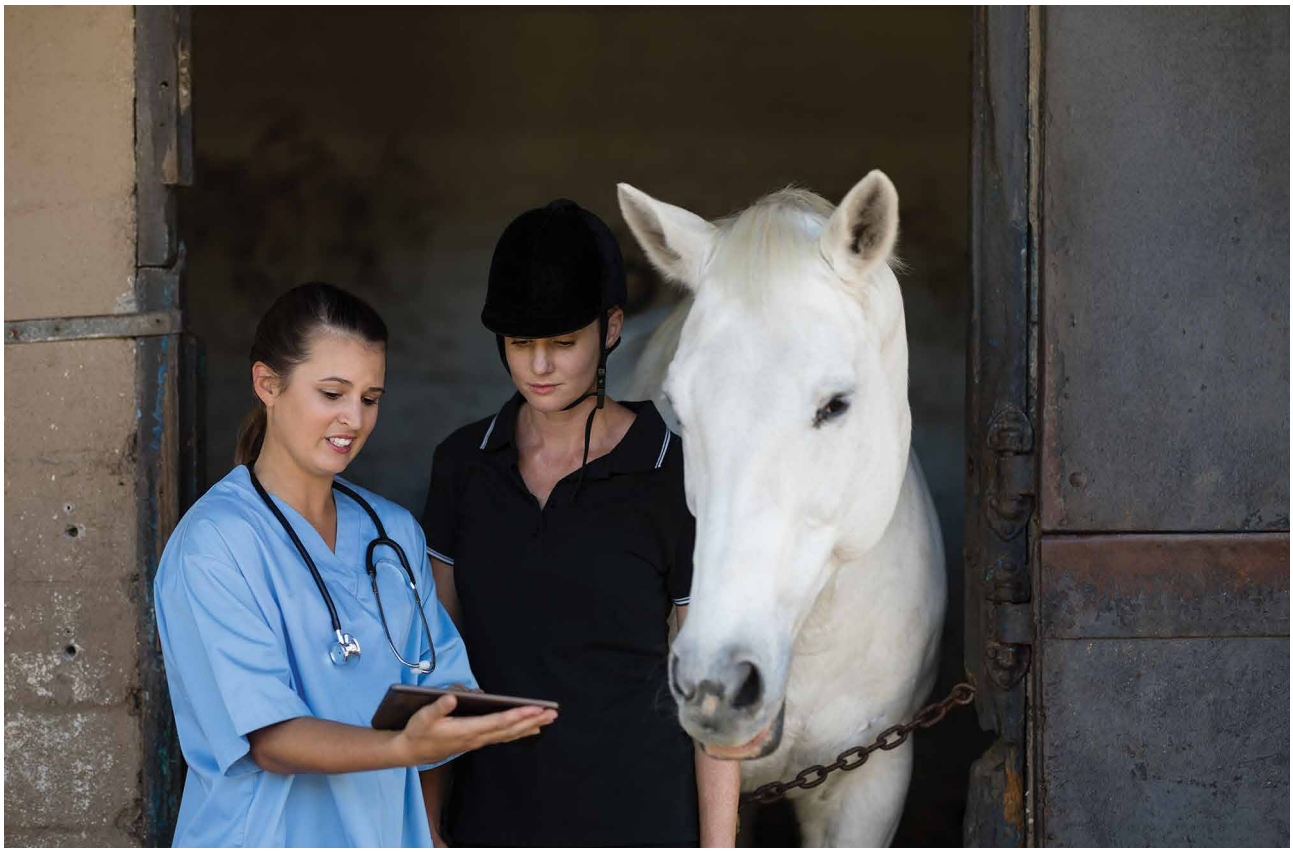


early 2014, when Hauck was introduced to Dr Martin K. Nielsen, a professor and renowned equine parasitologist at the Gluck Equine Research Center at the University of Kentucky. It was then that he came to realise the unmet need for improved diagnostics in clinical parasitology. The two lamented that although the faecal egg count was essential to modern veterinary medicine, the procedure had remained largely unchanged since its origin in the 1930s. The pair brought the problem to the attention to Dr Slusarewicz, whose experience in protein chemistry and analytical assay development was essential for addressing the problem.

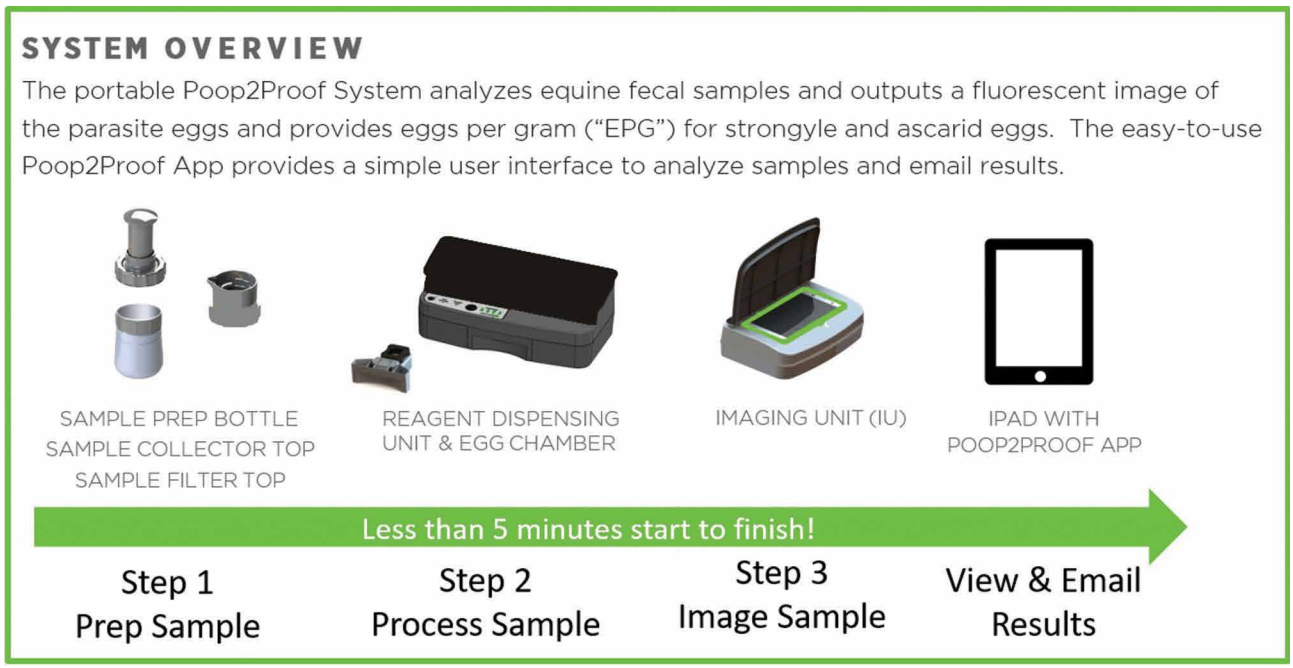
The trio founded MEP Equine Solutions and began research in June 2014 to tackle the challenge of modernising the faecal egg count. ‘We envision a more sensible and precise method for faecal egg counting that is more convenient for veterinarians and animal owners,’ says Dr Slusarewicz. ‘With this method, a microscope will no longer be needed, and the identification of eggs will not depend on the subjectivity of the person performing the test. This will all make it easy for everyone to do the right thing, by testing instead of treating prophylactically, and so prevent the ever-growing problem of parasite drug resistance.’

A Universal Marker for Parasite Eggs

Dr Slusarewicz realised that in order to develop an effective stall-side test for parasitic worms, he would need to find a better way of detecting helminth worm eggs in faecal matter. Calling on his training as a biochemist, he realised that the most efficient way to accomplish this goal would be to identify a compound that was ideally unique to egg casings and shared between multiple worm families – a universal egg marker.

Faecal matter often contains plant matter in various stages of digestion, along with mammalian cells from the host’s intestinal tract, and it would be critical to use a compound that would easily distinguish these components from the worm eggs. He settled on a carbohydrate called chitin as a key candidate for a universal egg marker that could be used in diagnostic procedures. Chitin is not found in either plant or mammal cells, but is a near universal component of insect exoskeletons, fungi and egg casings. With this feature in mind, Dr Slusarewicz and his colleagues chose chitin towards developing a next generation faecal egg counting protocol.

The team began by developing a biochemical tag, by attaching fluorescent dye to a protein that binds to chitin. Once they confirmed that the dye would attach itself to chitin molecules, they needed to confirm how effective the dye would be under the intended usage conditions. To do so, they went to the University of Kentucky Maine Chance Research Farm and local animal shelters to obtain faecal samples from horses, cattle, goats, sheep, dogs and cats. The team split each sample, with one half



used for a standard faecal egg count and the other half reserved to be tested with the team's new protocol. They then stained the faecal matter with the dye and viewed it under a fluorescent microscope. To their delight, the dye illuminated eggs from multiple species of helminth worm, making visualisation and counting much easier than with the classical method.

Harnessing the Technology in Your Pocket

The next phase the process was to make the counting process easier. The traditional method involves a laboratory technician performing the count, and requires an expensive microscope, a technician with specialised training, and a significant amount of time. Dr Slusarewicz and his colleagues wanted to investigate if the counting could be automated, and further, if it could be done using less expensive and more common equipment – such as a smartphone.

Dr Slusarewicz and a team of engineers built a specialised cradle that a smartphone can be placed into, to create consistent high-quality images of the dye-stained eggs. The cradle incorporates a high-magnification lens attachment and a light source to make the eggs glow, which focuses onto a grid where treated faecal samples can be loaded. With this setup, it became relatively easy to capture high-quality images of the fluorescent eggs, which can then be used in counts.

To reduce the time and technical expertise needed for a count, Dr Slusarewicz's next goal was to develop an image-processing program that could perform the egg counts automatically. To ensure that the program would not accidentally count other potential chitin-containing materials, such as fungal spores or fragments of injected insects, the team selected parameters designed to single out worm eggs.

Initially, these automated counts were performed by importing images onto a computer, but the team took the process a step further and developed a smartphone app – with the ability to do the same counts without transferring the image to a new device. With the combination of the photo cradle and app, the team was now able to use a single smartphone to image and analyse a sample.

To account for the various generations of smartphone technology in circulation, the team tested the setup with multiple phones of varying photographic strength. The results were astounding – even with the lowest resolution mobile camera, the rig was able to provide faecal egg counts consistent with and often more accurate than counts performed by a trained technician. Further, the app was able to distinguish between two different types of common and most pathogenic equine helminth worm eggs – strongyle eggs and ascarid eggs.

A New Vision for Parasite Detection and Treatment

The work of Dr Slusarewicz, the team at MEP Equine Solutions and the University of Kentucky, could prove to be transformative to the way helminth worm parasite infections are diagnosed and treated worldwide. A procedure that once took days to produce results can now be performed with startling accuracy in under 5 minutes out in the field by any veterinarian with a smartphone, which sends an email with the photo and record of the results to both the veterinarian and animal owner. Rather than applying monthly rotations of de-wormers to an entire barn full of horses and hoping for the best, horse owners can now provide targeted treatment that fully addresses the unique needs of a specific animal. Treatments may easily be tailored to a particular horse's parasitic load, shedding status, and the types of worms the animal is infected with. Horses will be spared unnecessary drug administration, and strategic application of de-wormers in infected animals will reduce the rates of drug resistance in helminth parasites.

The MEP team plans to expand the system's reach beyond the needs of the equine community, to revolutionise parasite diagnostics across multiple species and medical needs, possibly even to humans. Their next research directions involve refining the accuracy of their technology to identify more types of helminth worm eggs and produce a more precise laboratory version that runs on a desktop computer. The team has made amazing strides in parasite diagnostics over the past three years, and envision even greater advances in the years to come.

Meet the researcher

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Paul Slusarewicz began his research career at the University of London, where he obtained both his BSc and PhD in Biochemistry. His thesis and his postdoctoral work focused on the identification and purification of various proteins involved in transporting material around a cell. From there, he transitioned into industrial biotechnology and pharmaceutical R&D, quickly developing from a research scientist into an experienced leader, gaining expertise in product development and marketing. After successfully holding numerous high-level positions in various biotech firms, in 2014 Dr Slusarewicz co-founded his own start-up, MEP Equine Solutions LLC, in order to commercialise his invention of a novel quantitative assay for parasite egg load in faecal matter, where he currently serves as the Chief Scientific Officer. In addition to helping run his company, Dr Slusarewicz is an Adjunct Associate Professor in the Gluck Equine Research Center at the University of Kentucky.

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REFERENCES

P Slusarewicz, S Pagano, C Mills, G Popa, KM Chow, M Mendenhall, et al., Automated parasite faecal egg counting using fluorescence labelling, smartphone image capture and computational image analysis, International Journal for Parasitology, 2016, 46, 485–493.