UNDER THE MICROSCOPE

Microscope Use and Pathogen Identification in Birds and Reptiles

Dr Danny Brown
BVSc (Hons) BSc (Hons)
MACVSc (Avian Health)

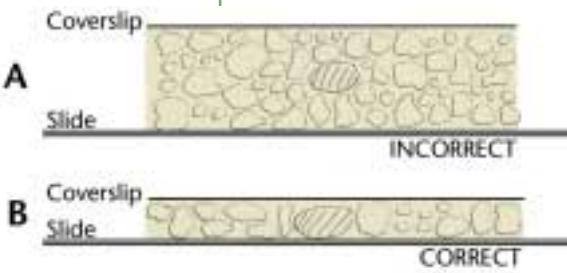
CONTENTS

AVIAN PATHOGENS

REPTILE PATHOGENS

ARTIFACTS/PSEUDOPARASITES

underneath (bottom piece of bread), a thin single layer of cheese (the sample) and a coverslip (piece of bread) on top. If we were able to look through the top surface (coverslip/top piece of bread) we should see one layer of finely distributed sample (cheese) and the bottom surface (slide/bottom piece of bread). What we do not want to see is the sample so thick and multilayered that, when viewed from above, allows us to see the top layer of the sample (cheese) but nothing underneath (subsequent layers of cheese). If this happens, we have no idea what is present in the subsequent layers. Other samples such as vomitus or oral mucous may be examined in the same way.

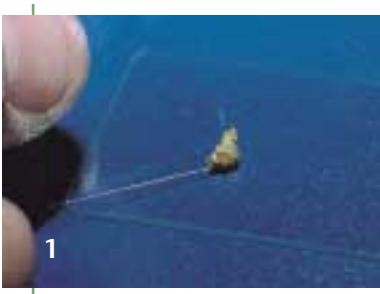


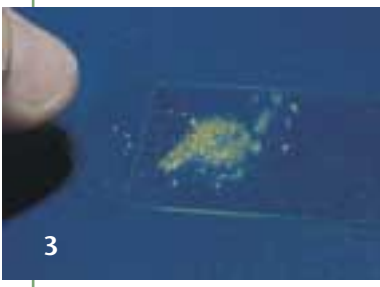
A: An excessively thick or multilayered slide sample may obscure the item being viewed.
B: A monolayer presents a finely distributed slide sample for clear visual examination.

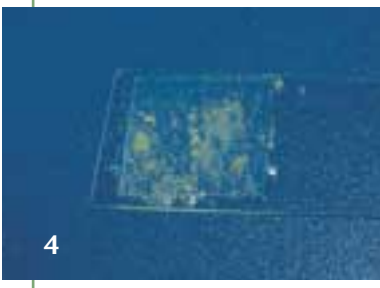
In order to achieve this ideal monolayer sample we must dilute and suspend the sample in a thin layer of liquid. The ideal liquid for all samples is lightly warmed saline solution (as used in intravenous fluid bags). This is not disruptive to any cellular membranes and will encourage survival of even the most fragile organisms. Bottled or tank water is the next best choice. Tap water will suffice for most situations but the chemicals in it will often rapidly kill sensitive pathogens such as protozoan flagellates.

The technique I use for preparing a sample for examination is as follows:

1. Collect a small piece of characteristic faecal material (or whatever you are examining). The usual amount is enough to cover one side of a matchstick head (or about 1/25 of a coverslip surface area). Pick this up with the corner of the coverslip or any firm object and place it in the centre of one end of the slide.
2. Using an eyedropper, dropper bottle, syringe or the tip of your finger, place one drop of diluting fluid (saline/water) onto the top of the small faecal sample on the slide.
3. Use the edge of the coverslip to mix the sample and the diluting fluid to a 'weak soup' consistency.
4. Place the coverslip over the mixed drop and allow the sample to spread out. If done correctly there should be just enough fluid to allow the sample to spread out under the coverslip without applying any pressure on it. If not mixed properly, or if there is non-organic material in the sample such as sand particles, the coverslip will sit up on one or more edges allowing air to become trapped under the slide.
5. Manipulate the coverslip with your finger, using gentle pressure in the centre until most air bubbles and any gritty material have been pushed to the side of the coverslip. Your coverslip should now cover a drop of liquid with a single layer of suspended material.



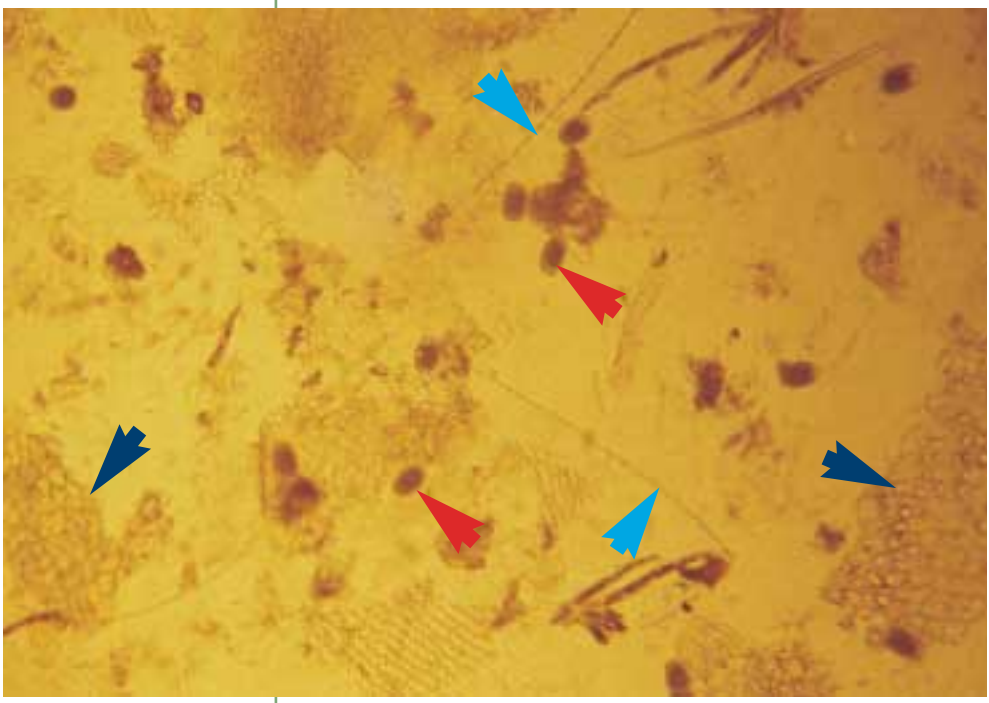




Interpretation of Results

Using a microscope correctly is only half of the battle. The most difficult point for most people to understand and appreciate is that a faecal sample contains more than just what we are looking for. It contains all of the waste from the gastrointestinal tract including undigested food, digestive bacteria, plant material, insect parts, feather and hair remnants and anything that is pathogenic. Your first view down a microscope will reveal any or all of these in a single view. The hard part is knowing what to ignore. All this extra material is background material in which you need to find a specifically shaped organism.

The pictorial section which follows will not only show what the most common pathogens look like but will also show what to expect in background material, including some confusing structures that we like to refer to as 'pseudoparasites' (because they are often mistaken for parasites). The pictorial examples will deal mainly with parasitic diseases in birds and reptiles as well as some fungal infections in birds. These are split into Avian and Reptile sections to avoid confusion. Also included are some examples of Gram stained bacteria. They are included here for demonstration purposes, so that you will understand what your veterinarian means by Gram-positive and Gram-negative infections. (See also *Special Techniques*, page 12)



Red arrows:
Roundworm egg.

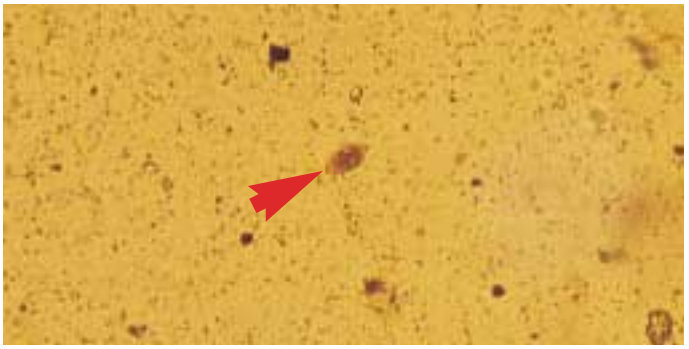
Light blue arrows:
Feather fragments.

Dark blue arrows:
Plant cell walls.

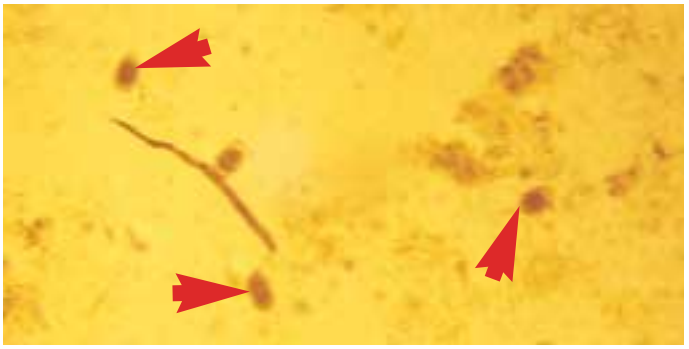
Many birds and reptiles in the wild have parasitic burdens but never develop a parasite-related disease. This is because it is unlikely that they are able to build up the numbers of parasites in the wild that they can develop in captivity. For example, a finch or

Roundworms (Ascarids)

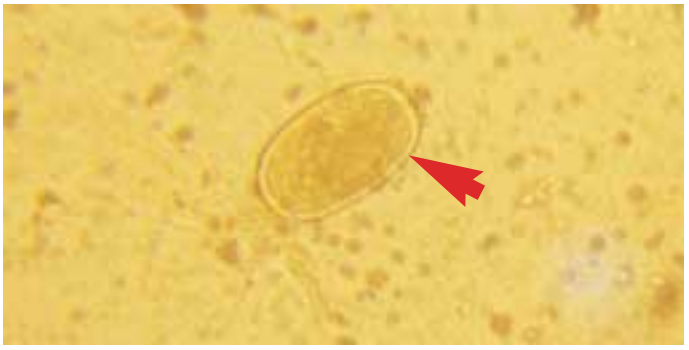
Generally, the oval-shaped eggs are large and thick-walled. The mottled central area is generally plumper and rounder in shape. They are likely to be the largest parasite eggs you will see. Found mainly in parrots, pigeons and poultry.



Roundworm egg (40X)—sample from a Princess Parrot—light burden.



Roundworm eggs (40X)—sample from a Budgerigar—heavy burden.



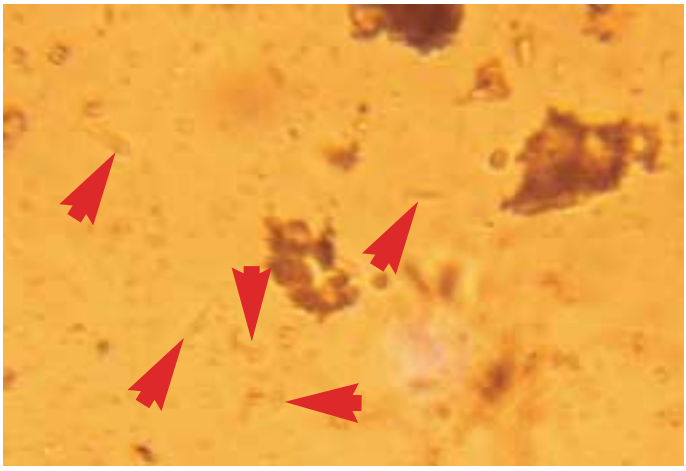
Roundworm egg (100X)—sample from a Princess Parrot.

Roundworm egg (100X)—sample from a Budgerigar.

REPTILE PATHOGENS

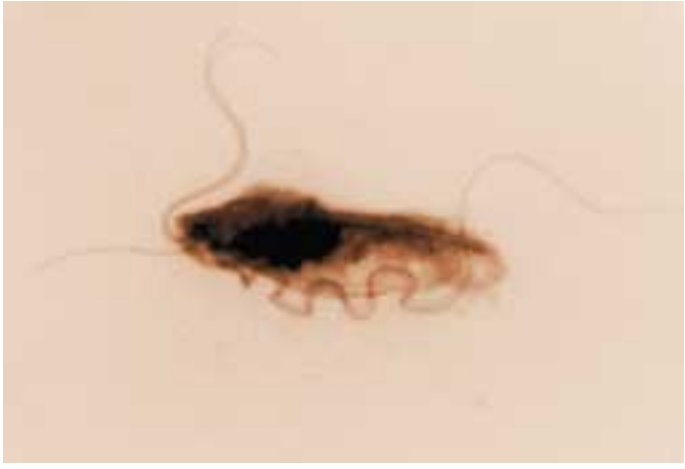
Motile flagellate protozoa (*Trichomonas* and other species)

All of these organisms have several features in common and appear similar under light microscopy. They have a circular to teardrop-shaped body along which are attached a series of waving appendages (flagella). The site of attachment of these flagella (front, back or midline) can be used as a rough guide to distinguish the species. These flagella are whipped around and propel the organism rapidly across the microscope field of view. Depending on the position of the flagella they will spiral, spin, rotate, gyrate, rocket or move like a falling leaf. They are very small and are difficult to see under less than 400X magnification. They also stop moving and die quickly once the faecal sample cools. Fresh warm faeces are best for identifying these organisms. There is little difference in the appearance of these organisms in both birds and reptiles. To get an appreciation of these organisms, collect and prepare a fresh warm dropping from a common household fence (or garden) skink. These commonly have very high natural flagellate burdens.



Motile flagellate protozoa (400X)—sample from a Stripe-tailed Monitor (See also page 32).

Red arrows: Flagellate protozoa organisms.



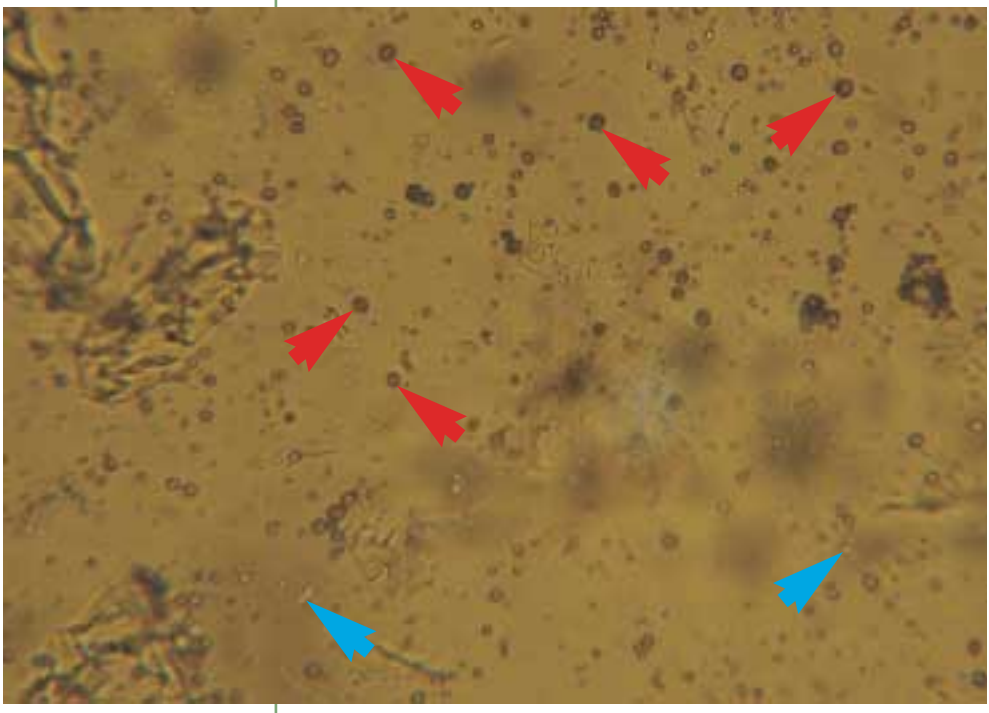
Motile flagellate protozoa (1800X)—sample from a Tiger Snake. This highly magnified view shows in detail the features of these organisms. The waving flagella should be visible at 400–1000X magnification.

ARTIFACTS/ PSEUDO-PARASITES

Knowing what to ignore in a sample is as important as knowing what to identify. The following views portray many of the more common items that are often misidentified as parasites due to similarity of shape, regularity of their appearance or high density on slides (See *Interpretation of Results* on page 18). Both avian and reptile pseudoparasites will be identified as many of these are common to both types of faecal examinations.

Urate crystals

Both birds and reptiles excrete nitrogenous waste in the form of urates (the white part of the droppings). Understandably, this often contaminates faecal samples. The individual crystals may be seen in massive amounts. They appear as circular structures, often with an internal crystalline or star pattern. They have no cell wall and will vary in size. They are most often mistaken for coccidia.



Urate crystals (100X).

Red arrows:
Urate crystals.

Light blue arrows:
Budding yeast organisms.