Case study: Follicular stasis in a Spur-thighed Tortoise, Testudo graeca

Anorexia is one of the most common presenting conditions in chelonia seen in general practice (Goodman 2009; Divers 2001). It is a clinical sign and not a disease and can be associated with husbandry problems and with almost any acute or chronic disease (McArthur 2004). It is essential that a diagnosis is achieved in addition to treating the clinical signs. In this case, plasma biochemistry and haematology, faecal analysis, survey radiographs and ultrasonography were performed as part of the diagnostic work up.

Key words: tortoise, Testudo graeca, anorexia, follicular stasis

Signalment
A seventy year old, female, spur-thighed tortoise (Testudo graeca) was kept in an enclosure with a ceramic heat lamp and a full spectrum UVA and UVB light. The owner had never kept any other tortoises with her and had owned this animal for fifty years.

Clinical history
The tortoise presented in September with a history of prolonged anorexia, lethargy and lack of faecal production. The usual diet consisted of a variety of edible garden plants, weeds, flowers and grasses (dandelions, plantain, mallows, sow thistle, chickweed, nasturtium flowers, campanula, lavatera and sedum) as well as dark leafy greens. No additional supplements were used.

Physical exam / differentials
Clinical examination was unremarkable with the exception of hind limb paresis. Due to the history and clinical examination, the tortoise was admitted for further diagnostic tests to investigate for any underlying causes. From the history one of the differential diagnoses was reproductive disease such as follicular stasis. Follicular stasis is also known as preovulatory egg binding, and is a common reproductive problem seen frequently in captive lizards and tortoises. Follicular stasis describes a condition where ovarian follicle development is static, resorption of the follicles do not occur, and over time inflammation of the follicles and ovary frequently occurs. Other differentials included: dystocia, impaction, renal and/or hepatic insufficiency, organomegaly causing impingement on the gastro-intestinal tract, or neoplasia.

Diagnostic procedures
Haematology and biochemistry
These were assessed at Pinmoore Animal Laboratory Services (PALS). The skin of the neck was swabbed and blood was collected in a lithium heparin tube using a 25 gauge needle from the right jugular vein. There was a marked leukopenia 0.3x10⁹/l (1-4.5) which is a common finding in a debilitated tortoise and is often a feature of immunosuppression and chronic disease. Common biochemistry changes in reptiles with follicular stasis include hypertriglyceridaemia, hypercalcæmia, hyperphosphataemia and hyperalbuminaemia. These derangements are all attributable to vitellogenesis, the accumulation of yolk in the maturing follicle. Oestrogen stimulates the liver to convert lipid to vitellogenin which is then selectively absorbed from the bloodstream by the follicles (Denardo, 2006). If the animal is presented later in the disease process, bloods may show hypoaalbuminaemia due to prolonged anorexia. In this case, there were increases in total calcium 4.94mmol/l (1.65-3.30), albumin 33g/L (13-26) and total protein 51g/L (23-44).

Parasitology
To rule out impaction and debilitation from a heavy burden of internal parasites, a fresh faecal sample was collected from the tortoise and a wet mount was examined. A small amount of faeces was mixed with saturated zinc solution and left to stand for ten minutes with a cover slide to be examined for nematode ova. A small number of oxyurids were observed which was deemed a normal finding in a tortoise. Oxyurids are frequently found and for the most part have developed a commensal relationship with their host. They have...
even been suggested to have beneficial effects preventing constipation (Telford 1971). Other publications suggest that in high numbers, anorexia (Martinez-Silvestre 2011) and death have occurred (Frank, 1981). In captivity, parasite burdens can rapidly increase due to space restricted housing and reduced host immunocompetency, (Schneller, 2008). Reinfection by the faeco-oral route can commonly occur if the environmental factors are not addressed. In this case, only a small number of oxyurids were observed and treatment was not deemed necessary. Environmental cleaning to prevent further re-infection and repeat testing in six months was advised.

**Radiography**

Radiography was performed to investigate whether eggs were present within the coelom. The standard three views (dorsoventral, lateral and craniocaudal were taken). In chelonia, follicles are rarely visualised on radiographs as a consequence of the shell. In other reptiles (especially lizards) follicles can be visualised as bunches of oval, soft tissue structures. Radiographs in this case were unremarkable with no eggs visible.

**Ultrasoundography**

Ultrasoundography (Figure 1) using a high frequency transducer (7.5MHz) was used to examine the patient via the prefemoral fossa and the cervicobrachial windows. Ultrasoundography is useful to monitor follicular development and, in this case revealed numerous follicles of varying sizes. The follicles were visualised as hyperechoic, spherical, homogenous structures that filled a significant part of the coelomic cavity (Hochleithner et al. 2015). Publications on ultrasonographic assessment of normal annular cycles in reptiles are limited (McArthur 2004). The presence of moderate numbers of follicles during late autumn and spring is considered a normal finding. However, in Mediterranean tortoises finding excessive numbers of medium (5–15mm diameter) and large (15–22mm) follicles is strongly suggestive of follicular stasis. Vitellogenic follicles can be up to 42mm in giant species of chelonia (Hochleithner et al. 2015). Sequential ultrasonography over a period of several weeks showing 20–50 mature follicles without evidence of regression is suggestive of follicular stasis (McArthur 2004).

**Computed tomography**

Computed tomography (CT) imaging can be considered for certain cases as it has advantages over both radiography and ultrasonography, allowing assessment of the number, size, form, contents and density of follicles and eggs. In this case study diagnosis was achieved by ultrasonography but CT (Figure 2) would be useful in larger chelonia such as Sulcatas (Geochelone/ Centrochelys sulcata) and Aldabran tortoises (Aldabrachelys gigantea) where ultrasonography of the prefemoral fossa would be impossible without sedation.

**Endoscopic examination**

Endoscopic examination, with entry into the prefemoral fossa in an anaesthetised tortoise will allow direct visualisation of the follicles (Divers 1999). This method can be used to confirm diagnosis and also to perform endoscope-assisted oophorectomy (Divers, 2014). In this case, confirmation of diagnosis by endoscopy and endoscope-assisted surgery were declined.

**Supportive care/therapeutics**

Fluids and nutritional support were given. Warm fluids were administered by gavage via a feeding tube at a maintenance rate suggested by Kirchgressner and Mitchell (2009) of 30ml/kg/24hours divided into separate feeds. The tortoise was bathed twice daily in warm water for ten minutes to stimulate urination and defaecation and to enable fluid absorption via the cloaca, as described by McArthur (2004). After twenty-four hours of fluid therapy nutritional support was provided via a feeding tube using a complete herbivorous diet (Emeraid Herbivore, Lafeber International). This provides semi-elemental nutrition that replenishes the animal’s depleted energy, fat and protein stores, provided in a format which is readily absorbed, assimilated and metabolised by the patient and can be administered via fine gavage feeding or pharyngostomy tubes.

**Surgery**

After the initial stabilisation period the tortoise underwent surgery to remove the follicles. Alfaxalone (Alfaxan; Jurox) was administered by a slow intravenous injection to effect, via the jugular vein to induce