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Short Communications

Clinical pathology of hypocalcaemia in adult grey parrots (*Psittacus e erithacus*)

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CALCIUM exists in three forms in avian serum: as an ionised salt, bound to proteins and in a complex bound to a variety of anions (citrate, bicarbonate and phosphate). The majority of veterinary pathology laboratories report a total calcium concentration, measured by spectrophotometer, which reflects the total combined levels of ionised calcium, protein-bound calcium and complexed calcium (Bush 1991). Measurement of total calcium in a bird with abnormal protein levels would not truly reflect its calcium status, as any changes in serum albumin values will affect the total calcium concentration, leading to an imprecise result. For example, in laying female birds, serum albumin levels may rise by up to 100 per cent to provide albumin for yolk and albumen production (Williams and others 2001). A blood sample analysed at this time would show an inflated total calcium concentration due to an increased protein-bound calcium fraction, while the ionised calcium level would not be affected. The binding reaction between the calcium ion and albumin is strongly dependent on pH, so acid-base imbalances would be expected to affect concentrations of ionised calcium. Therefore, an individual with metabolic acidosis would be expected to show ionised hypercalcaemia due to decreased protein binding. With an alkalotic individual, ionised hypocalcaemia would occur as the protein-binding reaction increases. Thus, the measurement of ionised calcium provides a more clinically relevant indication of an individual's calcium status, especially in diseased animals (Portale 1999).

Heparin binds calcium, which is a potential problem when analysing avian blood samples, because it is the normal anticoagulant used. Each unit of heparin has been demonstrated to bind 0·001 mmol/l of ionised calcium (Fraser and others 1994). It is therefore important to achieve the correct ratio of heparin anticoagulant to blood volume by filling the blood sample tubes with the required amount. EDTA anticoagulant should be avoided for ionised calcium assays due to calcium chelation. Blood samples for ionised calcium assays should be analysed as soon as possible after venepuncture, as changes in the pH of the sample will affect the accuracy of the ionised calcium levels measured. Glycolysis by the red blood cells will continue to produce lactic acid as a by-product, reducing the pH of the stored sample; however, chilling the sample after venepuncture will reduce this effect. The sample will lose carbon dioxide if it is exposed to room air, increasing the pH of the sample and subsequently reducing the amount of ionised calcium measured. Despite this, a study in dogs suggested that samples would not be adversely affected if not assayed for up to 72 hours, so it is possible to use external laboratories (Schenck and others 1995).

The metabolites of vitamin D_3 are structurally identical across all species, so assays for human 1,25-dihydroxycholecalciferol and 25-hydroxycholecalciferol can also be used in birds (Hollis and others 1999). The measurement of 25-hydroxycholecalciferol is considered to be the best assessment of vitamin D_3 status in any

individual because it has a longer half-life than other vitamin D_3 metabolites (Hollis and others 1999). The concentration of 25-hydroxycholecalciferol correlates well with dietary vitamin D_3 intake or exposure to UVB light (Soares and others 1995). Recently, ELISAs for 25-hydroxycholecalciferol have been developed with the advantages of both convenience and economy over traditional radioimmunoassays. Sample handling for vitamin D_3 assays is not critical and assays are available for both plasma and serum samples. Samples do not need to be frozen before analysis, because the vitamin is stable at room temperature. Repeated freeze-thaw cycles of the sample should be avoided, however, as the metabolite will denature.

Hypoparathyroidism or hyperparathyroidism can be diagnosed from direct measurement of parathyroid hormone (PTH). PTH circulates as a mixture of intact hormone and inactive mid-region and carboxyl terminal fragments. These fragments have long half-lives and interfere with intact PTH assays, so the majority of human assays involve a two-site radioimmunoassay (Blind and Gagel 1999). Most human assays concentrate on the mid and terminal segments of the PTH molecule due to the very short half-life of the biologically active 1-34N sections. It has been demonstrated that in chickens PTH consists of 88 amino acids with significant gene deletions and insertions compared with the mammalian homologue, although its molecular weight is similar (Russell and Sherwood 1989). Unfortunately, correlation in structure between the poultry and mammalian PTH molecules is very poor in the middle and terminal sections, so PTH assays have traditionally been difficult in birds. The avian and mammalian PTH molecules have greatest homology in the biologically active 1-34N regions. PTH is extremely labile at room temperature, and any assay requires exacting sample handling to produce good results (Barber and others 1993).

Hypocalcaemia is a common clinical presentation in captive adult grey parrots (*Psittacus e erithacus*), with clinical signs ranging from ataxia to seizures (McDonald 1988, Hochleithner 1989). It has been postulated that the condition is due to nutritional secondary hyperparathyroidism related to the feeding of seed diets with low calcium and vitamin D_3 contents (Klasing 1998). This study examined the clinical pathology of 19 adult grey parrots presenting with neurological signs consistent with hypocalcaemia.

A 2 ml blood sample was taken from the brachial vein of 19 adult grey parrots exhibiting clinical signs of hypocalcaemia, under isoflurane anaesthesia, as part of the routine investigation, and divided equally into heparin and EDTA Eppendorf tubes. The EDTA sample was centrifuged for 15 minutes and the plasma titrated into another Eppendorf tube. The plasma sample was immediately cooled to -70°C for subsequent analysis for PTH. The heparinised sample from each bird was analysed for ionised calcium, total calcium and 25-hydroxycholecalciferol, in addition to a routine avian blood profile. Plasma was analysed for 1,25-dihydroxycholecalciferol in 10 birds and PTH (PTH 1-34N) in five birds.

Ionised calcium concentrations were determined using an AVL 9181 analyser (Roche Diagnostics) within 30 minutes of venepuncture in order to avoid potential problems associated with any delay in electrolyte assays. The methodology employed by analysers to assay ionised calcium is based on the ion-selective electrode measurement principle to determine individual ion values precisely. An enzyme immunoassay (OCTEIA 25-hydroxycholecalciferol kit; IDS) was used for the quantitative determination of 25-hydroxycholecalciferol in serum and plasma. The 1,25-dihydroxycholecalciferol level was determined by radioimmunoassay by Vet Med Labor, Munich, Germany. PTH was assayed using a competitive enzyme immunoassay kit specifically designed to detect PTH 1-34N (Peninsula Laboratories). Unfortunately, it was not possible to use this assay routinely in all clinical cases due to the large sample size required (1 ml whole blood volume per assay) and the complexity of the assay.

Table 1 shows the results for calcium parameters in the hypocalcaemic birds. In all cases, a low plasma ionised calcium concentration was demonstrated, compared with the published reference ranges for parrots. In five of the 19 cases, despite a low plasma ionised calcium concentration, total calcium concentrations were within the normal range. This might be expected, especially in female birds, due to fluctuating protein concentrations during the egg-laying cycle. The failure to measure ionised calcium could lead to misinterpretation of calcium results in birds, as any change in protein-bound calcium is not thought to have any pathophysiological significance (Torrance 1995). It is considered of utmost importance when investigating abnormalities of calcium metabolism in psittacine birds to assay ionised calcium.

View this table: [in this window] [in a new window]	**TABLE 1 : Results of clinical pathology tests in adult grey parrots (*Psittacus e erithacus)* exhibiting clinical signs of hypocalcaemia**

A low 25-hydroxycholecalciferol concentration was detected in all the birds, compared with the reference range published for poultry. Low levels of 25-hydroxycholecalciferol suggest poor storage of vitamin D_3 in the birds, predisposing them to hypocalcaemia, due to a failure to provide adequate vitamin D_3 in the diet or a lack of UVB light exposure. The values were also below the reference ranges published for psittacine birds (Stanford 2003). The measurement of 1,25-dihydroxycholecalciferol has not been performed previously in psittacine birds, but all the results were below the reference range for poultry, which may be indicative of a failure in vitamin D_3 metabolism in grey parrot (Dacke 2000).

The measurement of PTH suggested high circulating concentrations in the five birds assayed compared with poultry reference ranges, suggesting that the clinical signs of hypocalcaemia in grey parrots are due to nutritional secondary hyperparathyroidism.

All the 19 birds made a full recovery on a standard treatment protocol of 10 mg/kg parenteral calcium (calcium borogluconate 5 per cent) once daily, diet improvement (Harrison's High Potency Course formulated diet) and UVB light supplementation (Arcadia 2·4% UVB lighting) for 12 hours daily.

This study suggests that the measurement of both 25-hydroxycholecalciferol and 1,25-dihydroxycholecalciferol is useful and of clinical significance in grey parrots. Assays for these metabolites are becoming more affordable and should perhaps form part of any routine investigation of calcium metabolism disorders. As it becomes feasible to measure vitamin D_3 receptors in birds, further research could be carried out in grey parrots to investigate whether they have a congenital vitamin D_3 receptor resistance. PTH assays require further research in grey parrots, and as these assays are validated and become more economic, the measurement of PTH could also be useful in this species.

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