RADIOGRAPHIC MEASUREMENTS FOR PDD DIAGNOSIS IN SPIX'S MACAWS (*CYANOSPITTA SPIXII*) AT AL WABRA WILDLIFE PRESERVATION (AWWP), QATAR

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Summary

Proventricular dilatation disease (PDD) is characterised by weight loss, physical weakness, regurgitation and/or neurological signs. PDD is a serious problem for the population of Spix's macaws (Cyanopsitta spixii) at Al Wabra Wildlife Preservation (AWWP), Doha, Qatar. During their annual health checks, radiographs are taken in accordance with the official health programme guidelines for the species. Radiograph image measurements of proventriculus diameter, hourglass waist, liver width and keel height enable calculation of the proventriculus diameter-to-keel height ratio which might be a useful indicator of a birds PDD status. In addition, the change in these values from 2004 to 2007 was evaluated. From the measurements of proventriculus width, we did observe a trend of increased large proventricular diameters in positive birds, lower in birds suspicious for PDD and lowest in birds with no obvious PDD lesions, as diagnosed by crop biopsy. Proventriculus diameter-to-keel height ratio did not differ significantly between positive and non positive birds (classified by crop biopsy), but reflected a similar trend to proventriculus width. Therefore, radiographic proventricular diameter measurements might be a useful initial indicator for carrying out crop biopsies in birds to detect PDD. From January 2007 to September 2007, there was a significant decrease of proventriculus diameter, hourglass waist and liver width that was interpreted as an effect of significant dietary changes instigated in 2005 and further refined in 2007.

Introduction

Proventriculus dilatation disease (PDD) was first described in 1971 (GRAHAM, 1991). The disease is characterised by clinical symptoms such as weight loss, weakness, regurgitation, passage of undigested food in the faeces and/or neurological signs (PHALEN, 1986; HAMMER et al., 2005). Suspicions based on these clinical signs need to be corroborated by additional investigations. Possible confirmatory diagnostics include radiography, barium-contrast-studies, endoscopy and biopsies of the crop, proventriculus, or ventriculus (PHALEN, 1986). Typical findings are a distention of the proventriculus and an increased transit time of barium in serial radiographs, ulceration and impaction on endoscopic examination (PHALEN, 1986, GREGORY et al., 1994), and the detection of a nonpurulent ganglionitis in the crop, characterised by lymphocytic or lymphoplasmacytic infiltration in the myenteric plexus, by histologic examination of crop biopsies (BOND et al., 1993; GREGORY et al., 1996). The aetiology of PDD was presumed to be Avian Paramyxovirus serotype 1 (APMV-1) (GRUND et al., 1999), but recently 3 research groups have claimed that Avian Bornavirus (ABV) could be the causative agent and can be detected with PCR testing of tissue of the brain, adrenal glands and gastrointestinal tract (KISTLER et al., 2008; HONKAVUORI et al., 2008). In addition, a new method of

radiographic diagnosis, using the proventriculus diameter-to-keel height ratio, has been introduced in the last year (DENNISON et al., 2008) which seems to be a practical, non-invasive and easily applied way to identify PDD-cases antemortem.
PDD is a serious problem in the population of Spix's macaws in Al Wabra Wildlife Preservation, Doha, Qatar (DEB et al., 2008). The Spix's macaw (*Cyanopsitta spixii*) is the only member of the genus *Cyanopsitta* and has been presumed extinct in the wild since the year 2000, due to loss of habitat and illegal trapping (DEL HOYO et al., 1997). At the time of writing, there were 63 individuals in the International studbook managed captive population, distributed in Brazil, Switzerland, Tenerife and the Al Wabra Wildlife Preservation (AWWP), Qatar. Fourty-seven animals are held at the Al Wabra Wildlife Preservation (AWWP) where 16 new individuals have been bred so far (WATSON et al., 2007). Intensive preventive health management of Spix's macaws is an important tool in the conservation effort for this species. Very little reference data is available for the species, with the exception of blood reference values which have been published recently (FOLDENAUER et al., 2007). During the annual health check that each Spix's macaw at AWWP undergoes, a radiographic examination is done along with - in case of a special indication - a crop biopsy and/or endoscopy of the proventriculus. In an earlier report, the applicability of serum diagnostics for AMPV-1 in diagnosing PDD was tested (DEB et al., 2008). Crop biopsy with subsequent evaluation has been considered the gold standard (GREGORY, 1996). Henceforth, the words "negative", "positive" and "suspicious" refer to the histological diagnosis. Here, the results of the three methods - crop biopsy, endoscopy and x-ray-evaluation - were compared with respect to the diagnosis of PDD.

Material and methods

Altogether, 149 radiographs were evaluated from 56 Spix's Macaws that lived at AWWP between 2004 and 2007. The health examination of Spix's macaws at AWWP was performed under general anaesthesia. For this procedure, the birds were anesthetised with isoflurane in oxygen (induction: 5 % isoflurane in 1 l/min oxygen, maintenance: 2 - 2.5 % isoflurane in 1 l/min oxygen). Blood samples and radiographs were routinely taken. All radiographs of the Spix's macaws were taken with a commercial x-ray unit (Gierth HF100 ultraleicht plus) and film (Factor 100x). The focal-film distance was 75 cm and the exposure time 0.08 sec (40 kV). Birds were radiographed both in laterolateral and ventrodorsal projection. All radiographs were taken whilst the birds were still unconscious from the anaesthesia.
The width of the proventriculus at its widest point, the hourglass waist and the liver width and the keel height were measured and the proventriculus diameter-to-keel height ratio was calculated according to DENNISON et al. (2008). The data was compared with the results of crop biopsies and endoscopy. A positive result in crop biopsy is characterised by a non-purulent ganglionitis, a suspicious result by perivascular lymphoplasmacellular infiltrates and a negative result shows none of them. Differences between negative and positive or suspicious groups, respectively, were tested by independent sample t-test or ANOVA (and subsequent Bonferroni post hoc test) using SPSS 17.0 (SPSS Inc., Chicago, IL). In addition, an evaluation of the development of proventriculus diameter, hourglass waist and liver width over the years 2004 to 2007 was performed. Exclusion causes were radiographs with a low quality, if the proventriculus was superimpositioned by other structures or if the proventriculus had the design of a triangle at the junction of proventriculus and ventriculus.

Results and discussion

Negative cases based on crop biopsy (n = 40) did not differ significantly in proventriculus diameter (9.8 ± 1.3 mm) from cases labelled as suspicious (n = 10) (10 ± 0.9 mm), in the width of the hourglass waist (negative: 14.4 ± 2.8 mm, suspicious: 14.7 ± 2.7 mm) and the liver width (negative: 24.5 ± 3.5 mm, suspicious: 25.4 ± 2.2 mm). However, definitely positive cases (n = 7) did show a greater, but not significantly, proventriculus diameter (10.5 ± 1.7 mm, hourglass waist width 14.4 ± 2.4 mm, liver 24.9 ± 3mm) than negative cases (figure 1). Dividing all cases into 2 groups, one showing non-purulent ganglionitis (positive cases) and one without ganglionitis (negative and suspicious cases), there was seen a similar behaviour as mentioned above: A proventriculus diameter of 9.9 ± 1.1 mm (hourglass waist 14.6 ± 2.8 mm, liver width 24.9 ± 2.9 mm) for negative and suspicious cases and for positive cases a proventricular diameter of 10.5 ± 1.7 mm (hourglass waist 14.4 ± 2.4 mm, liver width 24.9 ± 3 mm), not differing significantly.
The observation of proventriculus during endoscopies was documented 15 times between 2004 and 2007. Out of these 15 cases, six birds showed an enlarged proventriculus, one with a positive, one with a suspicious, one with a negative result in crop biopsy and three without any crop biopsy result. Similarly, of the nine cases with a negative endoscopy result, four were tested negative in crop biopsy, too, one positive and four birds did not have any crop biopsy result. There were no significant differences seen between the negative and the suspicious group, but the trend that negative cases had smaller proventriculus diameter, hourglass waist and liver width than suspicious birds. Cases judged as negative by endoscopy (n = 9) had, on x-ray, a proventriculus diameter of 10.1 ± 1.3 mm (hourglass waist 13.6 ± 2.6 mm, liver width 23.7 ± 2.4 mm), and cases judged as suspicious by endoscopy (n = 6) had a proventriculus diameter of 10.8 ± 1.0 mm (hourglass waist 15.2 ± 1.5 mm; liver width 26.8 ± 2.7 mm) on x-ray (figure 2).

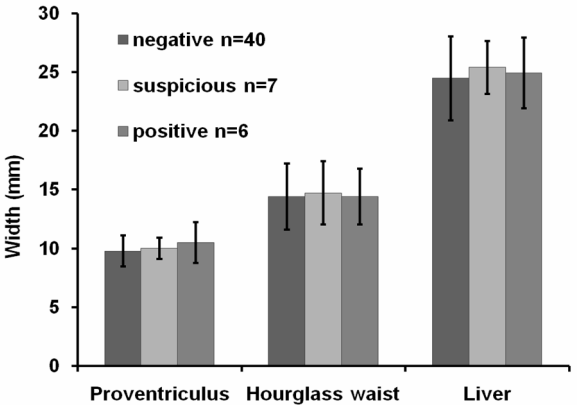

Figure 1: Radiographic measurements of proventriculus, hourglass waist and liver width with reference to the histology of crop biopsy.

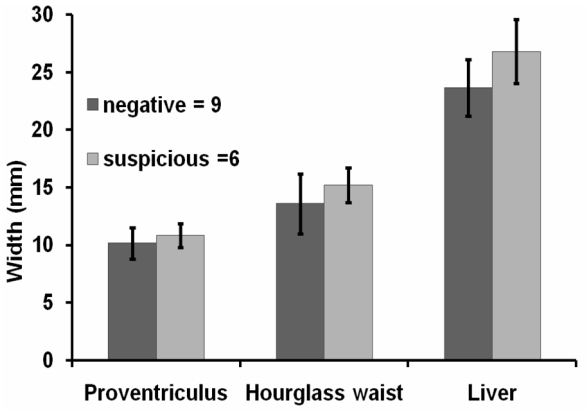

Figure 2: Radiographic measurements of the proventriculus diameter, hourglass waist and liver width on the reference to endoscopy results.

In a new method recently published (DENNISON et al., 2008) other measurements were taken than those in our study. For the proventricular diameter-to-keel height ratio a sensitivity of 100 % and also a specificity of 100 % for diagnosing PDD was calculated. This method seems to be more effective than simple measuring proventriculus diameter, hourglass waist and liver width. However, the calculation of

the proventricular diameter-to-keel height ratio of the Spix's macaws in AWWP did not show significantly different results in negative, suspicious and positive birds (figure 3). The ratio for negative birds (n = 21) was 0.40 ± 0.06, for suspicious birds (n = 5) 0.48 ± 0.04, and for the positive birds (n = 3) 0.54 ± 0.06 (p = 0.074, ANOVA). Two of the 3 positive cases were above the range (0.52) described as pathologic by DENNISON et al. (2008) but also 1 suspicious case and 3 cases with a negative crop biopsy result showed this attribute. Because the statistical result points towards a trend of increasing ratios for positive birds as compared with negative ones, the lack of statistical significance in the result could be a result of low sample size. However, although at this time the reliability of radiographic measurements in diagnosing PDD cannot be established, the potential for this non-invasive technique to be used as a screening tool for indicating further diagnostics like crop biopsies cannot be ignored.

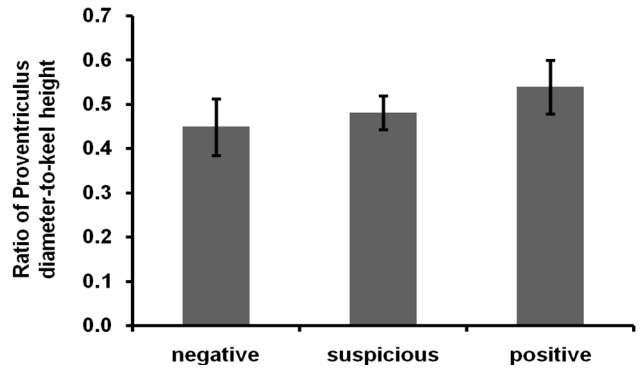

Figure 3: Ratio of Proventriculus diameter-to-keel height of negative, suspicious and positive PDD-cases in reference to histology of crop biopsy.

When looking at the development of the proventriculus diameter, hourglass waist and liver width over the years, a decrease of all these values from January 2007 to September/October 2007 in all individuals was detected, whereas before this period, values were only fluctuating within a constant range (figure 4). The proventriculus diameter decreased from 10.4 ± 1.0 mm (January 2007) to 9.5 ± 1.4 mm (September/October 2007), the hourglass waist from 15.5 ± 2.5 mm to 12.8 ± 2.0 mm and the liver width from 26.1 ± 1.6 mm to 23.4 ± 2.5 mm. The hypothesis was stated that either the focal film distance had been accidentally increased in the later measurements, or that the reduction in parameters actually represented the effect of a change in management. The first hypothesis was discarded by measuring the heart silhouette, which did not decrease in the same manner in the same time period (from 19.9 ± 1.4 mm in January 2007 to 19.5 ± 1.3 mm in September 2007). Therefore, the second hypothesis appeared more likely to be true.

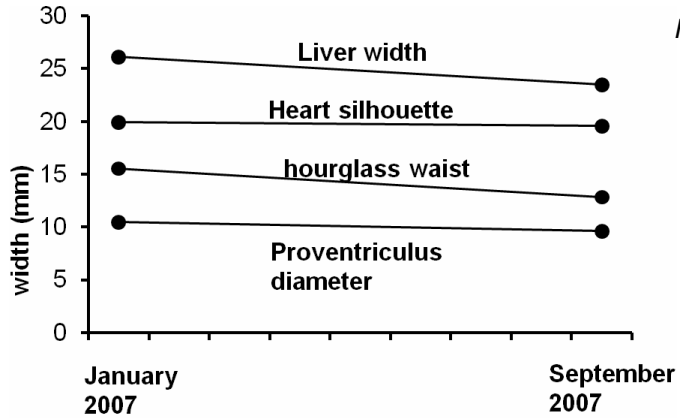

Figure 4: Development of proventriculus diameter, hourglass waist and liver width in comparaison with the heart silhouette from January 2007 to September 2007.

From July 2005 on, the feeding regime for the Spix' macaws was changed from an ad libitum offer of all food components (facilitating a selective intake of particularly high-energy feeds) to a restricted feeding where each bird has to ingest its complete diet (including the less-preferred, lower-energy seeds, sprouts, and pelleted feed). Additionally, some smaller changes to the dietary regime (a change of the pelleted diet component) were made until now. It was suspected that this feeding change had a positive effect on the muscle tonus of the gastrointestinal tract and the cause for the observed decrease in proventriculus diameter, hourglass waist and liver width in the last year.
The results of the radiographic measurements indicate that radiography with evaluation of the proventriculus diameter can be a useful non-invasive tool, wherein birds with increased radiographic proventricular diameters or proventricular diameter-to-keel height ratio should be subject to crop biopsy for confirmation of PDD. Even crop biopsies do not result in a definitive diagnosis of PDD because there is no significant relationship between the presence of lesions in the crop and the intensity of lesions in the proventriculus or ventriculus (GREGORY et al., 1996). Invasive diagnostic methods like proventricular and ventricular biopsy are procedures with higher risks (BOND et al., 1993), but must be still considered the most reliable diagnostic tool when screening for PDD (GREGORY et al., 1996). However, until serum and PCR diagnostics for avian bornavirus (KISTLER et al., 2008; HONKAVUORI et al., 2008) prove to be more reliable for testing PDD, these methods will remain the norm in routine diagnosis of PDD.

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References

BOND MW, DOWNS D, WOLF S (1993): Screening for psittacine proventricular dilatation syndrome. Proc Annu Conf Assoc Av Vet 92 - 97.

*DEB A, BORJAL RJ, BÜRKLE M, WATSON R, HAMMER S (2008): Evaluation of Avian Paramyxovirus-1 serology and crop biopsy for the diagnosis of proventricular dilatation disease in captive Spix's macaw (Cyanopsitta spixii). Scientific meeting of European Association of Zoo- and Wildlife Veterinarians (EAZWV) **7**, 239 - 242.*

DEL HOYO J, ELLIOT A, SARGATAL J (1997): Handbook of the Birds of the World. Barcelona: Lynx Edicions.

*DENNISON SE, PAUL-MURPHY JR, ADAMS WM (2008): Radiographic determination of proventricular diameter in psittacine birds. J Am Vet Med Assoc **232**, 709 - 714.*

*FOLDENAUER U, BORJAL RJ, DEB A, ARIF A, TAHA AS, WATSON RW, STEINMETZ H, BÜRKLE M, HAMMER S (2007): Hematologic and plasma biochemical values of Spix's macaws (Cyanopsitta spixii). Journal of Avian Medicine and Surgery **21**, 275 - 282.*

GRAHAM DL (1991): Wasting/Proventricular dilatation disease: a pathologist's view. Proc Annu Conf Assoc Av Vet 43 - 44.

*GREGORY CHR, LATIMER KS, NIAGRO FD, RITCHIE BW, CAMPAGNOLI R, NORTON TN, MCMANAMON R, GREENACRE CHB (1994): A review of proventricular dilatation syndrome. J Assoc Av Vet **8**, 69 - 75.*

*GREGORY CHR, LATIMER KS, CAMPAGNOLI RP, RITCHIE BW (1996): Histologic evaluation of the crop for diagnosis of proventricular dilatation syndrome in psittacine birds J Vet Diagn Invest **8**, 76 - 80.*

GRUND CH, GRIMM F, KÖSTERS J (1999): Serological studies on persistent APMV-1 infection associated with PDD. Proc Annu Conf Assoc Av Vet 19 - 23.

*HAMMER S, GERLACH H, BÜRKLE M, SCHULZ J (2005): Proventricular dilatation disease (PDD) in Spix's macaws (Cyanopsitta spixii). International Symposium on Diseases of Zoo and Wild Animals. Verh ber Erkrg **42**, 7 – 10.*

*HONKAVUORI KS, SHIVAPRASAD HL, WILLIAMS BL, QUAN PHL, HORNIG M, STREET C, PALACIOS G, HUTCHISON SK, FRANCA M, EGHOLM M, BRIESE T, LIPKIN WI (2008): Novel Bornavirus in psittacine birds with proventricular dilatation disease. Emerg Infect Dis **14**, 1883 - 1886.*

*KISTLER AL, GANCZ A, CLUBB S, SKEWES-COX P, FISCHER K, SORBER K, CHIU CL, LUBLIN A, MECHANI S, FARNOUSHI Y, GRENINGER A, WEN CC, KARLENE SB, GANEM D, DERISI JL, (2008): Recovery of divergent avian bornaviruses from cases of proventricular dilatation disease: Identification of a candidate etiologic agent. Virology Journal **5**, 88.*

PHALEN DN (1986): An outbreak of psittacine proventricular dilatation syndrome (PPDS) in a private collection of birds and an atypical form of PPDS in a nanday conure. Proc Annu Conf Assoc Av Vet 27 - 34.

WATSON R, DEB A, HAMMER S (2007): managing the world's largest population of Spix's macaws (Cyanopsitta spixii). Proceedings of the 33rd Annual Conference of the American Federation of Aviculture, LA, USA 118 - 132.