AN OUTBREAK OF PROVENTRICULAR DILATATION DISEASE IN PSITTACINE BREEDING FARM IN ISRAEL

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Summary
Proventricular dilatation disease (PDD) is reported in Israel for the first time following an outbreak in a psittacine breeding farm, in which the most affected species were the large parrots, Macaws and Grey Parrots. The diagnosis of PDD was carried out by a general pathological examination of the birds, with histopathological confirmation in some of them. The histological confirmation was suggestive of a viral disease associated with lesions in the nerve plexi of the gastrointestinal organs that impairs their normal absorptive function.

Introduction
Proventricular dilatation disease (PDD) is a fatal disease of birds characterized by a lymphocytic, plasmacytic inflammatory process of the central and peripheral nervous tissues. The most frequently affected are ganglia supplying the musculature of the digestive tract, causing atrophy of the smooth muscles of the crop, proventriculus, ventriculus and small intestine (Myenteric Ganglionisris), resulting in motility disorders and dilatation of the proventriculus (1). The disease was first described in the late 1970’s in Macaws imported into the United States and Germany as a malabsorption/maligestion disorder named Macaw Wasting Disease (2, 3, 4). In the order Psittaciformes, PDD has been reported in over 50 species, while the syndrome has also been reported in several other orders (5). In a study of almost 130 birds diagnosed with PDD, the affected age ranged from 10 weeks to 17 years (mean of 3.8 years) (6).

The most common clinical signs of PDD are depression, weight loss, regurgitation and indigestion. Central nervous signs may also be observed if central and peripheral nervous tissues are involved (5). These signs may include ataxia, abnormal head movements, seizures and proprioceptive or motor deficits. Clinical PDD is not easy to confirm in the live bird because X-ray imaging does not give a clear diagnosis and serology in this disease is lacking (7). Ultrasonographic demonstration of dilatation and impaction of the proventriculus and endoscopic examination that may demonstrate impaction, ulceration, and dilatation of the proventriculus, assist in PDD diagnosis (6). Additionally, a crop biopsy for the typical histopathological lesions can be performed, but all these findings are inconclusive.

Even though the suspected etiology of PDD seems to be viral (7, 8), no virus had been defined so far, and multiple viruses have been proposed for the syndrome, such as adeno-like virus, herpesvirus (Pacheco’s disease virus), polyomavirus, encephalitis, and paramyxovirus-like (5, 9). Up-to-date, a definitive diagnosis of PDD in dead birds requires the demonstration of characteristic lymphoplasmacytic infiltrates within nervous tissue, especially in the proventriculus, but also in the ventriculus, crop and brain. Nevertheless, histopathological findings are not always conclusive. Microscopic lesions were described by Gerlach (2) in only 62% of birds with typical clinical PDD. In contrast, 33% of the birds with lymphoplasmacytic proliferation in the proventriculus did not have proventricular dilatation. In a group of 15 psittacine birds with proventricular dilatation, typical lesions were found in the proventriculus of all birds, but only in 67% were lesions demonstrated in the crop (7).

Material and Methods
PDD in Israel - a case history
Sporadic unpublished cases of birds with clinical and pathological suspicion of PDD have been observed in the past in adult psittacines (especially Grey Parrots, Macaws and Eclectus) in various locations in Israel, but so far no consistent surveillance was conducted.

A severe outbreak in a psittacine breeding farm with high mortality that was diagnosed as PDD occurred in 2004 about one year after the introduction of a breeding pair of Blue and Gold Macaws (*Ara ararauna*). Most of the chicks revealed signs of PDD at the age of 10-14 wks, and one chick even at 30 wks. Forty-two Grey parrots (*Psittacus ethacus*) and eight Blue & Gold Macaws were hatched in this aviary in this period, of which about 27 birds died with typical lesions of PDD. In order to prevent further spreading of the disease, the owners agreed to euthanize the 18 clinically affected birds intended for breeding following the veterinarian's
about 27 birds died with typical lesions of PDD. In order to prevent further spreading of the disease, the owners agreed to euthanize the 18 clinically affected birds intended for breeding following the veterinarian’s advice. Several species remained free of clinical signs: Indian Ringneck Parrakeet (Psittacula krameri) (n=20), Jardine’s Parrot (Poicephalus guilelmi) (n=6), Pionus (Pionus menstruus) (n=6), Black-headed Caique (Pointes melanoccephala) (n=5), Sun Conure (Aratinga solstitialis) (n=3), and Lory (Tricholosus haematodus) (n=2), in addition to the 16 birds from the affected species that remained healthy.

The Macaws suspected to be the origin of this outbreak of PDD were transferred to a non-breeding aviary. All the premises where the affected birds were kept were disinfected with formaldehyde gas.

The distribution of morbidity and mortality of the various species in the breeding farm is presented in Table 1.

Table 1: Distribution of morbidity and mortality of various psittacine species in a breeding farm in which PDD occurred

<table>
<thead>
<tr>
<th>Species</th>
<th>N Healthy birds</th>
<th>Sick birds euthanized</th>
<th>Dead birds</th>
<th>% Affected birds (morbidity&amp;mortality)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grey Parrot</td>
<td>42</td>
<td>2</td>
<td>17</td>
<td>23%</td>
</tr>
<tr>
<td>Psittacus Elegans</td>
<td>4</td>
<td>0</td>
<td>1</td>
<td>25%</td>
</tr>
<tr>
<td>Blue &amp; Gold Macaw</td>
<td>6</td>
<td>0</td>
<td>1</td>
<td>16%</td>
</tr>
<tr>
<td>Ara Amarina</td>
<td>4</td>
<td>4</td>
<td>0</td>
<td>100%</td>
</tr>
<tr>
<td>Citron-Crested Cockatoo</td>
<td>4</td>
<td>4</td>
<td>0</td>
<td>100%</td>
</tr>
<tr>
<td>Cockatia Sulphurata</td>
<td>7</td>
<td>7</td>
<td>0</td>
<td>100%</td>
</tr>
<tr>
<td>Galah</td>
<td>3</td>
<td>3</td>
<td>0</td>
<td>100%</td>
</tr>
<tr>
<td>Eolophus roesepennis</td>
<td>3</td>
<td>3</td>
<td>0</td>
<td>100%</td>
</tr>
<tr>
<td>Ectlectus</td>
<td>3</td>
<td>3</td>
<td>0</td>
<td>100%</td>
</tr>
<tr>
<td>Echidnus rufus</td>
<td>3</td>
<td>3</td>
<td>0</td>
<td>100%</td>
</tr>
<tr>
<td>Total</td>
<td>69</td>
<td>16</td>
<td>18</td>
<td>35%</td>
</tr>
</tbody>
</table>

* Recovered after Celcox treatment (see below).
** One of these was treated with Celcox and died after 3 months.

As shown in Table 1, mortality rate was very high in Macaws, while morbidity was prominent in Grey Parrots. The other species had a lower incidence of morbidity and mortality.

Progression of the disease. In most cases the disease lasted for 2-10 days before death. The clinical signs were; stasis of the crop, refusal to eat pelleted food, refusal to eat spontaneously towards weaning time, and even refusal to accept the common amount of food by force-feeding, growth retardation, and in most cases, regurgitation. Three of the chicks had also central nervous signs (torticollis), one chick had bloody feces and bloody vomitus, and one bird had severe respiratory distress.

Pathological and histopathological lesions of affected birds.

Nineteen of the dead birds, most of them from the euthanized group (14 Grey Parrots, 3 Blue & Yellow Macaws, and 2 Citron-Crested Cockatoos), were necropsied for gross pathological evaluation and for comparison of crop versus proventriculus and brain lesions to test whether crop biopsy in the live bird is a reliable means of the clinical diagnosis of PDD (Bendheim et al. 10). Blood samples were taken before euthanasia for measuring serological and biochemical parameters.

Experimental treatment. All sick chicks except for 3 Grey Parrots were euthanized. The three remaining chicks were treated for 2 months with 10 mg per day of CELCOX 100, diluted in ENSURE-Plus (10 mg/10 ml), which belongs to a new family of non-steroidal anti-inflammatory drugs (NSAIIds) (1), and were caged elsewhere for follow-up for 3 months.

Results

Pathological lesions. At necropsy, a severe dilation of the proventriculus was found in all birds. Most of the birds were cachectic, had yellowish gelatinous content in the gastrointestinal tract, and had severe congestion of the blood vessels on the serosa of the proventriculus, ventriculus and small intestines. These birds also had hemorrhages on the skull, probably due to rapid head movements. Severe proventricular dilatation with congestion of blood vessels in serosa of proventriculus and ventriculus is shown in Fig. 1.

Histological lesions. Microscopic lesions were observed in 12 out of 19 cases (63%), in the brain, proventriculus or crop (in at least one of these organs). Multifocal mononuclear, lymphoplasmacytic infiltrates were noted in the ganglial area of the proventriculus (10/19) or the crop (4/19), and in a few cases in the intestines. Multifocal perivascular lymphoplasmacytic infiltrates and focal glosis were observed in the brain (cerebrum) (10/19). These lesions are typical of PDD and resemble the descriptions of others (7, 8).

Typical microscopic lesions visible in PDD-affected birds are presented in figures 2-5.

Differential diagnosis. Other diseases that may cause nervous signs were eliminated; Newcastle disease and avian influenza strain H9 (using hemagglutination inhibition), West Nile virus (virus neutralization in sera and PCR of brain tissue), bacterial and mycotic agents in brain, and Chlamydophila psittaci (using ImmunoComb Elisa of sera and immunofluorescence of organ smears).

Thirty-two birds from this outbreak and several other cases were examined for intra-crop fungi. Candida spp. (most of them were C albicans) were isolated from 11 of them, and Aspergillus fumigatus from one bird.

Biochemical parameters. Biochemical parameters considered as characteristic of digestive functions were measured in 11 birds using an autoanalyzer (Konelab 30i, Kone, Finland), and were compared with data published by Fudge (California Avian Laboratory, CA, 1998) for normal Grey Parrots (the predominant species in this outbreak). The results of the comparison are presented in Table 2.

Table 2. Comparison of biochemical parameters associated with digestive functions measured in blood of 11 euthanized birds with normal values published by Fudge (1998). Average values are given.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Normal birds</th>
<th>PDD-suspected birds</th>
</tr>
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<tbody>
<tr>
<td>Total protein (g/dl)</td>
<td>3.5</td>
<td>3.0</td>
</tr>
<tr>
<td>Albumin (g/dl)</td>
<td>1.6</td>
<td>1.2</td>
</tr>
<tr>
<td>Total calcium (mg/dl)</td>
<td>9.3</td>
<td>8.5</td>
</tr>
<tr>
<td>Alkaline phosphatase (U/L)</td>
<td>34</td>
<td>656</td>
</tr>
<tr>
<td>Amylase (U/L)</td>
<td>511</td>
<td>658</td>
</tr>
</tbody>
</table>
As it can be seen in Table 2, the values of total protein, albumin and total calcium in the PDD-suspected birds were slightly lower than in normal birds, while alkaline phosphatase and amylase, especially alkaline phosphatase were higher in these birds.

**Birds treated with Celcox.** All three birds treated with CELCOX 100 were symptomatically cured but one of them died after 3 months of therapy, with pathological lesions typical of PDD.

**Discussion**

This is the first report of an outbreak of proventriculit dilatation disease in psitacines in Israel. The outbreak was described in 10 to 30 wk-old chicks of 5 psitacine species with an average of 77% morbidity and mortality. The total mortality rate was high, (about 50%), and very high in Macaws (almost 90%), the species in which the first cases of PDD had been reported. The Grey Parrot is the species where most of the sick birds have been observed. Thus, at least according this outbreak these two psitacine species may be considered as the most affected ones.

To prevent further spreading of the disease, all the clinically affected birds were euthanized except for three Grey Parrots that were experimentally treated with non-steroidal, anti-inflammatory drug with relative success, as was reported also by Dahlhausen and Aldred (1).

All clinically affected birds had dilatation of the proventriculus; however, only 10 of 19 euthanized birds demonstrated typical histological changes in the brain or the proventriculus, and only 4 in the crop. Thus the brain and proventriculus may even present negative histological findings in birds with clinical PDD, as shown by others (2,11). The rest of the birds did not present any histological changes. An additional four birds from this outbreak with clinical PDD showed proventriculat dilatation at necropsy but had not been evaluated histologically. Crop biopsy, which is the most feasible surgical tool for diagnosis of PDD in live birds (7), cannot be recommended as the principal tool for PDD diagnosis, and more accurate means should be developed. According to others, antemortem evaluation of crop biopsy in PDD diagnosis may reach approximately 75% (12), however some samples may not contain affected nerve plexi and will give false-negative results (13). Taking additional specimens for histological evaluation is always recommended.

The chosen biochemical parameters, even though not statistically confirmed, indicate a tendency of impaired absorptive & digestive function. The decreases in total protein and albumin values and the slight decrease in calcium values in the affected birds are probably associated with reduced nutrient absorption due to the disorders in the gastrointestinal tract. The increase in alkaline phosphatase is probably associated with leakage from liver cells where the enzyme is produced, or with any gastrointestinal inflammation. The slight increase in amylase may be associated with some pancreatic impairment associated with malfunction of the gastrointestinal tract in PDD.

**References**

1. Dahlhausen, B. and Aldred, S. Resolution of clinical proventriculitis dilatation disease by cyclooxygenase 2 inhibition.


Fig. 1: Dilated proventriculus with blood vessels congestion on the serosal surface of a PDD-affected bird.

Fig. 2: Mononuclear lymphoplasmacytic infiltrates within a ganglion of proventriculus (X10).

Fig. 3: The ganglion area of Fig. 2, higher power (X40).

Fig. 4: Lymphoplasmacytic perivascular cuffing in the cerebral cortex (X50).

Fig. 5: Focal gliosis in brain (X50).