

# Pyrrrolizidine Alkaloid Toxicity

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## Abstract

Several cases of non-specific muscle weakness, depression and inappetence with or without anorexia were encountered in an avicultural breeding facility over six months. *In vivo* laboratory tests were inconclusive. Histological sections from autopsy material displayed evidence of pyrrolizidine alkaloid (PA) toxicity. Seed analysis was positive for PAs, two seed contaminants were found neither of which contained PAs. A case report and literature review are discussed.

## Case Report

The aviaries concerned are very well managed. None of the presented birds were new. They were not in contact with each other nor had there been any major movement or introductions within the facility. The affected birds were from different areas of the facility. No major management changes had been made except that a change in seed supplier had occurred in January-February 1993. The new merchant is held in high regard and supplies seed grown only on the Darling Downs.

### 22 March 1993

The first bird presented was a cock two year old Senegal Parrot. The clinical signs were acute onset, sick bird look (SBL), fluffed appearance, depression, inappetence, and disinterest in the environment. Examination showed a well-muscled bird with no discharges and was clinically normal on auscultation and palpation. Crop and faecal smears and Gram stains were normal. Bloods were taken for a body function. The bird was hospitalised with heat, antibiotics, and Ca EDTA. Intravenous (IV) fluids were administered. The condition of the bird improved within 24 hours. Bloods showed elevated liver enzymes and total bile acids, indicating liver disease (see Table 1). Treatment was continued for a further two days, then the bird was discharged on antibiotic therapy for a further 3 days.

### 13 July 1993

Derbyan hen 1 year old was presented. This bird showed the same non-specific clinical signs as with the Senegal but in addition was in a wasted physical condition. Auscultation and palpation were normal. Crop and faecal smears and Gram stains were normal. Radiographs were normal. Blood was taken for a body function and treatment was implemented as with the Senegal. Blood results indicated myopathy (see Table 1). The bird failed to respond after four days. Laparoscopy was performed and no abnormalities were detected.

Two days later the bird's appearance improved, she began to eat and was discharged.

### 20 July 1993

Three birds were presented with similar non-specific signs. They were in moderate to poor condition:

Split lutino alexandrine	SBL <sup>+</sup>
Derbyan cock	SBL <sup>++</sup>
Ringneck cock	SBL <sup>+++</sup>

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Treatment was instigated as before, i.e., antibiotics, chelation and fluids in addition to crop feeding with *Poly-Aid*<sup>®</sup>. Bloods were taken for body functions. The ringneck died overnight. Both other birds were improving. Blood results (see Table 1).

The ringneck apparently died of renal failure. The bird was sent to Veterinary Pathology Services (VPS) for autopsy. Histology of viscera and culture and sensitivity (C&S) of gut were requested. The histological findings were significant. See VPS form.

**Table 1**

	1	2	3	4	5
Function	Senegal	Derbyan	Derbyan	Alexandrine	Ringneck
Total bilirubin	5.8	8.1	7.1	6.3	2.0
AST	488.0	620.0	1150.0	2000.0	1600.0
Gamma GT	4.0	16.0	47.0	400.0	65.0
CPK	1572.0	8300.0	10,600.0	14,400.0	22,100.0
Cholesterol	4.4	-	-	-	-
Total bile acids	>40.0	-	-	-	-
Uric acid	0.5	0.8	1.4	0.8	5.6
EST WCC	18.0	16.0	12.6	21.0	10.6

#### VPS Form

##### Gross Pathology

The bird is in moderate flesh and minimal post mortem autolysis. There is some bending of the keel bone. The kidneys are swollen, pale tan in colour with numerous pale silver flecks. The ureters contain urate crystals. There is a mild haemorrhagic enteritis in the anterior intestine.

##### Histopathology

Sections of the lung show mild congestion. There are focal areas of inflammation consisting of macrophages and heterophils. The main region is associated with the larger airways. Sections of liver show widespread chronic inflammation of portal regions with fibrosis and infiltrate of lymphocytes and plasma cells with occasional heterophils. There is mild hyperplasia of biliary epithelium. Hepatocytes adjacent to areas of necrosis show mild megalocytosis and nucleomegaly. Eixed macrophages frequently contain hemosiderin. Sections of kidney show widespread tubular degeneration with urate accumulation and mild inflammation adjacent to crystal formation. Small number of renal tubular epithelial cells show cytomegaly.

##### Morphological Diagnosis

1. Nephrosis
2. Chronic toxic hepatitis
3. Acute renal tubular nephrosis with renal gout.

Samples of the remaining seed (budgerigar mix) were obtained. The client was advised to return to using his old seed supplier until the causative agent was discovered.

On examination of the budgerigar mix, three suspect seeds discovered.

1. A white millet-sized seed, smooth bright-yellow-cream and pea-shaped (approximately 5% of seed mix)
2. A furry olive bean-shaped seed intermediate in size to white millet and sorghum. (approximately 5 seeds per kilogram).
3. A black, hard pyramidal seed (approximately 5 seeds per kilogram).

The Type 1 seed was identified as hulled white millet. Type 2 seed was not identified by examination. Type 3 was identified as *Polygonum convolvulus* - commonly known in Queensland as buckwheat.

A sample of the mixed seed was sent for a standard toxicological screen at the Government Chemical Laboratories. The sample tested positive to pyrrolizidine alkaloids. The sample of seed Type 2 was delivered to the Herbarium for germination and identification on suspicion of being a *Crotalaria* spp. After about 6 weeks we received an identification of *Hibiscus trionicum*. This is, unfortunately, a non-toxic seed contaminant commonly found in Queensland.

The remaining batch of seed was sent for mycotoxin analysis to rule out aflatoxin contamination. These results were negative. This testing exhausted the remaining seed supply rendering further testing impossible.

### **Pyrrolizidine Alkaloids**

Plants containing PAs are common causes of poisoning of livestock worldwide. In Australia, numerous plant species contain PAs. The most important groups are:

1. *Crotalaria* (rattlepods)
2. *Senecio* (ragworts, fireweeds, groundsel)
3. *Heliotropium*
4. *Amsinckia* (iron weeds)
5. *Echium* (Patterson's curse)
6. *Trichodesma* (camel bush)

Of these 1 and 6 have broad toxicity, 2 is less toxic and 3, 4 and 5 are least toxic (Hooper, 1958). Recently, 100,000-200,000 chickens were poisoned in Australia by heliotrope seed contamination of feed (Gaul *et al.*, 1994).

PAs were first discovered in 1967 for *Senecio* spp. Many different PAs occur, and they broadly cause peracute to chronic disease. Often death occurs weeks to months after ingestion. The reproductive parts of the plants, including seeds contain the highest levels of PAs.

PAs are ingested in plant material and then metabolized by the liver to toxic metabolites, i.e., the **dihydroalkaloids**. These metabolites attack hepatocytes, causing necrosis. They are very reactive but relatively short lived.

Some circulate in the blood to the lungs where they become secondary metabolites, i.e., the **dehydroamino alcohols**. These are less reactive but are long-lived and have antimetabolic nucleotoxic effects (Kirschner, 1960). These compounds are carcinogenic, teratogenic and mutagenic.

The effects of PAs and their metabolites on tissues can be summarised by direct necrosis, inhibition of mitosis leading to megalocytosis or by direct action on vascular endothelium causing vascular damage and oedema.

## Specifically

### Liver

Most PAs cause primary liver disease. The effects on vascular endothelium causes haemorrhagic necrosis and veno-occlusive disease (This can occur within days of a single dose of PAs). The antimetabolic effects cause megalocytosis, cytoplasmic invaginations and intracytoplasmic inclusion bodies.

Portal fibrosis, bile duct proliferation, regeneration nodules and tumours are common.

### Lung

The primary change is an increase in the medial thickness of muscular pulmonary arteries. Sometimes an arteritis and destruction of the external elastic lamina occurs. These lesions result in pulmonary hypertension and a corresponding increase in right ventricular weight.

Secondary myocardial damage can also occur (Larranconi and Huxtable, 1987). Alveolar epithelialization and emphysema can occur (Hooper, 1978).

### Kidneys

PAs produce a mild to severe renal tubular necrosis. Megalocytosis follows this acute damage.

### Other Tissues

PAs exert their toxic effect on other tissues as well:

- Spongy degeneration of the cerebrum;
- Necrosis of pancreatic acinar cells (Mattocks, 1986);
- Gastrointestinal tract lesions (Hooper, 1978); and
- Vascular degeneration of the pancreas (Allen *et al*, 1970) and mesenteries (Schoental *et al*, 1959).

In poultry: Acute disease was characterised at autopsy by severely congested livers with haemorrhagic streaks and yellow to white areas on the liver surface and swollen gall bladders. Subacute affected birds showed petechial haemorrhages in the liver.

Chronic cases showed sub-capsular haemorrhagic cysts and congestion of hepatic blood vessels in addition to the other signs (Sergany *et al*, 1991).

## Microscopic Changes in Poultry

### Acute Cases

Areas of hepatocellular degeneration and necrosis predominate.

### Sub-acute and Chronic Cases

Coagulative necrosis of hepatocytes and bile duct hyperplasia with fibroplastic cellular proliferation are the most typical lesions.

No other organ changes were included in this assessment (Sergany *et al*, 1991).

## Carcinogenicity, Mutagenicity & Antimitotic Activity of PAs

Various PAs are carcinogenic, primarily to hepatocytes (Hirono, 1981; Hirono *et al*, 1983), but pancreatic and spinal cord tumours are common (Schoental *et al*, 1972). The carcinogen is generally a metabolite formed by the liver (Mattocks, 1986). Some PAs are being investigated as chemotherapeutic agents because of their antimitotic activity (Mattocks 1986). Studies have shown that all hepatotoxic PAs are also mutagenic. They have a prolonged effect at inhibiting DNA synthesis and hence liver regeneration after necrosis (Bull *et al*, 1968). PAs have also been found to be embryotoxic and teratogenic. (Mattocks, 1986).

## Discussion

Although a precise contaminant of the seed was not established, the history, autopsy findings, histological changes and presence of PAs in the sampled seed, and the absence of aflatoxins in the seed, are all firm grounds for suspecting PA toxicity. Unfortunately, no seed was available for further assessment for seed contaminants and also no further tissues were available for PA analysis. The purpose of this article is to draw awareness to PA toxicity and to suggest it be included in the differential diagnosis of hepatic disease, specifically aflatoxicosis.

## References

- Allen JR and Carstens LA (1970). Clinical signs and pathologic changes in *Crotalaria spectabilis* in intoxicated rats. *American Journal of Veterinary Research*, **31**: 1059.
- Bull LB, Cullvenor CCJ and Deck A (1968) The Pyrrolizidine Alkaloids, North Holland, Amsterdam.
- Gaul KL, Gallagher PF, Reyes D, Stasi S and Edgar J (1994). Poisonings in pigs and poultry by stock feed contaminated with heliotrope seed. In Plant Associated Toxins: agricultural, phytochemical and ecological aspects. Colgate AM, Darling PR, Allen SG, Huxtable CR, Mead RT and Pawter KE. CAB International Wallingford U.K.
- Hirono I (1981). Natural carcinogenic products of plant origin. *CRC Critical Reviews of Toxicology*, **8**: 235.
- Hirono I, Ueno I, Aiso S, Yamaji T and Haga M (1981). Carcinogenic activity of *Fargugium japonicum* and *Senecio cannabifolius*. *Camera Letters*, **20**: 1991.
- Hooper PT (1978). Pyrrolizidine alkaloid poisoning - pathology with particular references to differences in animal and plant species. In: Effects of poisonous plants on livestock. Eds Keeler RF, Van Kampen, KR and James LF. Academic Press, New York, 161.
- Kirschner HE (1960). Nature's healing grasses. HC White Publications, Riverside.
- Lafranconi M and Huxtable RJ (1981). Pyrrolizidine and the pulmonary vasculature. *Reviews of Drug metabolism and Drug Interaction*, **3**: 271.

Mattocks AR (1986). Chemistry and toxicity of pyrrolizidine alkaloids. Academic Press, London.

Schoental R and Cavanagh JB (1972). Brain and spinal cord tumour in rats treated with pyrrolizide alkaloids. *Journal of the National Cancer Institute*, **49**: 665.

Schoental R and Magee PN (1959). Further observation on the subacute and chronic liver changes in rats after a single dose of various pyrrolizidine (*Senecio*) alkaloids. *Journal of Pathology and Bacteriology*, **78**: 471.

Sergony MA, Morsy AA, Shazly MO and Sawy AM (1991). Pathological effect of *Senecio* plant in chickens. *Egyptian Journal of Comparative Pathology and Clinical Pathology*, **4**: 150.