SUSPECTED POISONING IN WILD GALAHS

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CASE REPORT

This report describes the pathology found in groups of wild galahs (*Eolophus roseicapilla*) from the Riverina, showing clinical signs of acute central nervous system disease during the winter or early springs of 2010 and 2011. Anecdotal reports were received of up to 150 birds on the Wagga Wagga Campus of Charles Sturt University either being found dead or in varying stages of nervous system dysfunction. Live affected birds submitted to the Veterinary Diagnostic Laboratory at Charles Sturt University were typically found depressed, in good body condition with clinical signs of ataxia and body tremors. Most failed to make any attempts to escape or fly when picked up. Bloods were collected from nine acutely affected birds and analysis demonstrated relatively normal haematology. All birds had moderate to marked elevation in AST and CK concentrations suggestive of muscle damage, possibly due to recumbency or due to handling. AST may also be elevated in liver disease and in bird 7, the elevation in GLDH compared with other birds suggested that hepatocellular damage might also have taken place. Birds 7-9 had mild increases in plasma uric acid concentrations likely to have been secondary to dehydration. Birds 3-5 also had mild hypoglycaemia. The significance of the increased amylase in Bird 1 is uncertain. These birds were treated symptomatically with either subcutaneous or *per os* fluids but only two birds made a full recovery. The others were necropsied along with all birds submitted dead.

Post mortem examinations demonstrated few consistent lesions but included moderate congestion of the liver and meninges. Many birds had crop and or ventricular contents with wheat and other seeds. Several birds had dark green discoloured contents within the ventriculus, duodenum and intestines. Samples of visceral organs and brain were collected into formalin for routine paraffin embedding and histopathological examination. Consistent findings were mild to moderate diffuse hepatic, cerebral and renal congestion. In the liver of two birds there were occasional eosinophilic intranuclear inclusions within otherwise normal hepatocytes. Ziehl–Neelsen staining of these to detect sequestered lead were negative. One bird had clinical signs and histological evidence of psittacine beak and feather disease. Another had defeathering of the skin covering the head associated with a wound and haemorrhage to one side of the occiput which covered a fracture of the skull. Histopathological examination of this lesion demonstrated severe acute heterophilic encephalitis and bacterial aggregations consistent with a penetrating wound from predation.

In June 2011 Defence confirmed that 326 deceased galahs were found and removed from the parks and gardens of RAAF Base Wagga. The following week a further 61 deceased galahs were also found and removed but only one bird was submitted for necropsy examination. As with the other cases described above this one was in excellent body condition and had non-specific gross and histological

lesions. Infectious disease was ruled out by bacteriology and microscopic examination and a presumptive diagnosis of suspected organophosphate intoxication was considered. Defence was conducting a mouse baiting program at RAAF Base Wagga to combat a recent mouse plague in the area but accidental exposure was considered highly unlikely given that the bait stations were inaccessible to birds and that there were no lesions consistent with such poisoning.

DISCUSSION

Birds have been long used as sentinels of environmental hazard in mines and recently deaths in wild birds provided an early health warning to widespread community lead exposure in Esperance, Western Australia, revealed by the unexpected deaths of thousands of wild birds (Gulson et al., 2009). This outbreak highlighted a deficiency of knowledge concerning the toxicological affects of lead within Australian ecosystems. In many Northern hemisphere biomes anthropogenic lead poisoning is considered a serious problem due to its persistence in the environment, its ability to affect multiple body systems including the behaviour of prey by altering central nervous function, and its bioaccumulation within food webs. Like other toxic heavy metal poisonings such as cadmium and arsenic, it has multisystemic physiological effects including chronic negative impacts on skeletal and reproductive systems, embryonic and nestling mortality and growth depression (Roux and Marra, 2007, Burger, 1995, Roodbergen et al., 2008, Wayland et al., 2008, Martinez-Lopez et al., 2006, Burger and Eichhorst, 2005).

A major objective in any investigation of a suspect acute toxicosis in wildlife is to rule out known possible causes that might explain the clinical signs and lesions (Raidal and Jaensch, 2006). Clinical signs of acute central nervous system dysfunction in wild birds are typically relatively non-specific and can be due to a wide range of infectious and non-infectious agents. During the summer of 2010-11 arbovirus infections have caused outbreaks of sometimes fatal encephalitis in horses throughout south eastern Australia and this has been attributed to massive increases in arthropod vectors secondary to widespread rainfall throughout the region. Flaviviruses such as *Murray valley encephalitis virus, Kunjin virus* and Alphaviruses such as *Ross river virus* are presumably relatively common infections in wild birds throughout Australia (Kay et al., 2007, Schmaljohn and McClain, 1996, Marshall et al., 1982, Liehne et al., 1976) but most are asymptomatic and few have been associated with nervous signs or typical lesions of non-suppurative encephalitis and multi-organ necrosis and inflammation as seen in North American and psittacine birds infected with West Nile virus (Himsworth et al., 2009, Palmieri et al., 2010, Stockman et al., 2010). However, the peak numbers of affected galahs occurred in winter when mosquito vectors are non-active or senescent. Furthermore, the lack of evidence of necrosis or inflammation suggestive of arbovirus infections or neurotropic, velogenic Newcastle disease made these or other viral infections (Staeheli et al., 2010, Ouyang et al., 2009, Weissenbock et al., 2009, Carranza et al., 1986) very unlikely as the cause of the mortalities.

Acute bacterial encephalitis was also considered a possibility for individual galahs and was confirmed in one bird but this individual case was most likely secondary to predation after it first became immobilised and susceptible to attack from ravens. *Chlamydia psittaci* infection can cause outbreaks of mortality associated with airsacculitis, hepatitis and splenitis but these lesions or microscopic evidence of *C. psittaci* infection were not detected. Likewise, systemic fungal infections were also ruled out by necropsy and histopathological examination. Parasitic infections such as haemoprotozoa and migrating nematodes are known causes of nervous disease in wild birds in Australia (Raidal and Jaensch, 2000, Montali et al., 2004, Gelis et al., 2011) but there was no similar evidence in the affected galahs.

Affected birds found on or near the Wagga Wagga campus of Charles Sturt University were found near agricultural land freshly sown with cereal crops treated with fluquinconazole (Jockey® Bayer CropScience Pty Ltd) and their gastrointestinal contents included ingested wheat seeds, an unnatural food source during winter. Ingestion of treated grain is therefore a possible explanation of the clinical signs noticed in the birds. According to the manufacturer, fluquinconazole is an S6 medication[1] with an oral LD50 in the rat of 429-2000 mg/kg and acute oral LD50 in bobwhite quail and mallard duck of > 2000 mg/kg. No data are available for Australian wildlife or psittacine birds. The chemical is toxic to fish and other aquatic organisms but a low hazard to birds and earthworms and crops grown from treated seed cannot be used for stockfeed plants for 8 (canola) to 12 (wheat) weeks after planting[1].

With current global sales approximating $1.8 billion *per annum* triazoles have grown to become the largest class of fungicides used in agriculture since the launch of triadimefon by Bayer in 1973. Numerous other compounds quickly followed including triadimenol, bitertanol and propiconazole. Current global usage includes prothiaconazole, tebuconazole, epoxiconazole, propiconazole, difenoconazole, flusilazole, tetraconazole, fluquinconazole and flutriafol. Along with the better known chemotherapeutic azole drugs such as miconazole, itraconazole, and clotrimazole they are broad spectrum, highly efficient antifungal agents which inhibit the enzyme cytochrome P450 14α-demethylase. This enzyme cleaves two methyl groups from lanosterol in the synthesis of ergosterol, a provitamin to Vitamin D2 which serves the same function of cholesterol in animal cells and is the major sterol component of yeast and fungal cell membranes. Ergosterol is also present in the cell membranes of some protists, thus providing the basis for antifungal usage to treat some protozoal infections. A different mechanism of action is behind the antifungal drug Amphotericin B which binds to cell wall-bound ergosterol within the fungal cell membrane creating a lytic pore.

Exposure to potentially neurotoxic organophosphate, carbamates and organochlorine based insecticides is recognised as a potential threat to wildlife but these compounds are typically applied in spring and summer to combat plague aggregations of flightless locust nymphs which pose a serious threat to agriculture (Fildes et al., 2009, Fildes et al., 2006, Hill, 1988, Kucera, 1987). Whilst the usage of organochlorine chemicals such as heptachlor and dieldrin is now banned in most parts of the world, the widespread use of these chemicals in pest control and agriculture from the 1950s along with their persistence in the environment still poses a significant long-term threat to wildlife. Continued illegal use may be adding to this. The highly lipid-soluble nature of these agents, combined with their environmental persistence, favours bioaccumulation and biomagnification in top predators (Henny et al., 2003, Senthilkumar et al., 2001) in food webs. Even low levels of exposure may impair immune function and have detrimental oestrogenising effects (Bustnes et al., 2004, Minh et al., 2002, Choi et al., 2001, Erikstad et al., 2011), but not acute nervous disease as seen in the affected galahs.

Exposure to cholinesterase-inhibiting organophosphate and carbamate insecticides and organochlorines as environmental pollutants can be assessed by plasma cholinesterase analysis (Fildes et al., 2009, Brasel et al., 2007, Martinez-Haro et al., 2007, Kiesau and Kummerfeld, 1998) and gas chromatography (Behrooz et al., 2009, Ricca et al., 2008, Papp et al., 2005, Rocque and Winker, 2004, Kunisue et al., 2003). These agrichemicals are widely used to prevent or treat parasitic infections in crops, and livestock. Fortunately, and unlike organochlorines, most do not accumulate

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[1] http://www.bayercropscience.com.au/cs/products/productdetails.asp?id=306

To download acrobat file:
http://www.bayercropscience.com.au/resources/products/msds/306-2243-Material%20Safety%20Data%20Sheet%20(MSDS).pdf

in fat deposits and are rapidly, almost completely, eliminated following ingestion or absorption. However, chlorinated organophosphates are more lipid soluble and therefore residues persist longer than other organophosphates.

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Table 1. Haematology Results Galahs (2010), CSU Campus

White Cell Parameters	No. 1	No. 2	No. 3	No. 4	No. 5	No.6	No. 7	No. 8	No. 9	Reference Range
WBC	10.68	7.72	13.8	19.52	19.84	13.4	7.24	10.56	10.6	2.0-24.8×10^9/L
Heterophils	9.7 (90.8%)	5.03 (65.1%)	9.6 (69.6%)	15.62 (80.0%)	15.44 (77.8%)	9.59 (71.6%)	3.91 (54%)	9.11 (86.3%)	5.96 (56.2%)	1.2-21.9×10^9/L (26-89%)
Lymphocytes	0.94 (8.77%)	2.29 (33.4%)	4.04 (29.3%)	3.42 (17.5%)	3.89 (19.6%)	2.79 (20.8%)	3.19 (44%)	1.29 (12.2%)	3.68 (34.7%)	0.2-7.3×10^9/L (6-61%)
Monocytes	0.03 (0.266%)	0.18 (1.14%)	0.03 (0.245%)	0.30 (1.53%)	0.43 (2.15%)	0.87 (6.47%)	0 (0%)	0.14 (1.37%)	0.86 (8.09%)	0-1.0×10^9/L (0-15%)
Eosinophils	0.01 (0.106%)	0.0 (0.043%)	0.00 (0.018%)	0.03 (0.14%)	0.00 (0.03%)	0.01 (0.07%)	0 (0%)	0.01 (0.069%)	0.00 (0.016%)	0-0.9×10^9/L (0-8%)
Basophils	0.01 (0.053%)	0.02 (0.29%)	0.11 (0.81%)	0.17 (0.89%)	0.01 (0.39%)	0.15 (1.09%)	0.14 (2%)	0.01 (0.052%)	0.11 (0.998%)	0-0.4×10^9/L (0-4%)
Red Cell Parameters										
RBC	4.30	4.17	3.96	3.95	4.40	3.86	3.62	3.84	3.52	**x 10^{12}/L**
HGB	233	246	241	238	242	225	201	235	219	**g/L**
HCT	0.60	0.628	0.607	0.593	0.612	0.561	0.508	0.565	0.555	**0.49-0.66 L/L**
MCV	140	151	153	150	139	0.145	140	147	158	**fL**
MCH	54.3	59	61.0	60.4	55.1	58.4	55.5	61.3	62.2	**pg**
MCHC	389	392	398	402	396	401	395	417	395	**g/L**
RDW	11.1	9.80	9.63	10.5	10.4	13.8	13.6	9.83	10.0	**%CV**
Thromobocytes	Adequate	Adequate	Adequate	Adequate	Adequate	Adequate	Adequate	Adequate	Adequate	

Table 2. Plasma Biochemistry Results Galahs (2010), CSU Campus

ANALYTE	No. 1	No. 2	No. 3	No. 4	No. 5	No. 6	No. 7	No. 8	No. 9	REFERENCE RANGES
Fasting Status										
Total Protein	26	30	33	29	31	24	**17**	**21**	31	22-58 g/L
Albumin	8	8	7	9	8	7	4	6	8	g/L
Globulin	19	22	26	20	23	18	13	15	24	g/L
A:G Ratio	0.42	0.36	0.27	0.45	0.35	0.39	0.31	0.4	0.33	
Uric Acid	10.5	4.1	3.3	5.7	6.4	2.2	**15.1**	**27.5**	**22.2**	2-11.57 mg/dl
AST	301	412	221	365	481	**215**	**3908**	**1050**		211-833 U/L
CK	**416**	**452**	**685**	**407**	**872**	**275**	**1175**	**1113**		47-270 U/L
GLDH	<1	<1	<1	<1	<1	<1	32	1	<1	U/L
GGT	<1	<1	<1	<1	2	<1	<1	<1	<1	U/L
Glucose	18.6	18.7	**14.8**	**15.7**	**15.9**	**15.5**	**12.0**	**15.3**		17-24 mmol/L
Amylase	1944	415	408	299	410	645	791	310		U/L
Sodium	139	143	142	144	142	145	145	143	142	mmol/L
Potassium	9.2	7.6	7.0	6.7	11.2	2.1	2.5	2.8	6.6	mmol/L
Na:K Ratio	15	19	20	22	13	68	58	50	22	
Chloride	105.8	106.4	104.7	106.2	105.8	101.1	101.0	101.9	104.1	mmol/L
Calcium	2.04	2.05	2.12	2.11	2.07	2.09	2.05	1.86	2.49	mmol/L
Phosphorus	0.92	0.71	0.86	0.96	0.58	1.23	1.33	0.58	1.52	mmol/L
Ca:Phos	2	3	2	2	4	2	2	3	2	
Cholesterol	6.9	7.7	7.0	6.1	7.8	5.6	7.1	6.9	**12.2**	3.2-11.6 mmol/L