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Clinical Communication

Acute photosensitisation and mortality in a herd of dairy cattle in Tasmania

HM Golder[*§], N Moss[*], G Rogers[†], B Jackson[‡], N Gannon[#], PTW Wong[¥] and IJ Lean[*]

Abstract

CASE HISTORY: A herd of Holstein, Jersey, or Holstein-Jersey cross lactating cattle of mixed ages presented with a sudden drop in milk yield in 94/678 cows on 3 October 2014 (Day 0). The herd was located in Gretna in the Derwent Valley (Tasmania, Australia) and had been grazing dryland pasture.

CLINICAL FINDINGS: On Day 0 the cows variably showed recumbency, peracute photosensitisation, inflamed coronary bands, conjunctival erythema, periauricular oedema, distress indicated by kicking at the flank, bruxism, discomfort, weight shifting, vocalisation indicating pain and depression. Blood samples collected on Day 4 from five clinically affected cows showed high activities of aspartate aminotransferase, glutamate dehydrogenase and gamma-glutamyl transferase. Morbidity, based on the number of treated cases within 72 hours of clinical onset, was estimated at 165/678 cows (24.3%). Mortality over the first 30 days was 19/678 cows (2.8%).

PATHOLOGICAL FINDINGS: Necropsies of two cows on Day 4 showed marked distension of the gall bladder and extensive icterus. Necropsies of another two cows on Day 5 showed enlarged livers with severe damage and oedema of the distal abomasum. Severe ulcerative abomasal gastritis was present in both cows. Hepatic histopathology was consistent with chronic cholangiohepatitis.

MYCOTOXICOLOGY: Fifty-five different mycotoxins were detected from a barley grass (*Hordeum murinum*) sample from the presumably contaminated pasture. Concentrations of B-trichothecenes, fumonisins, and zearalenone metabolites from this sample were remarkably high. The leaf smut, *Jamesdicksonia dactylidis*, that has not been previously reported in Tasmania, was identified from the sample of barley grass, but it is not known whether the smut can produce toxins.

DIAGNOSIS: Probably an undescribed peracute mycotoxicosis associated with the ingestion of contaminated dryland pasture.

CLINICAL RELEVANCE: A definitive diagnosis could not be reached in this case of acute photosensitisation and mortality in dairy cattle grazing possibly contaminated dryland pasture. The findings differed from both facial eczema and acute bovine liver disease, suggesting an undescribed mycotoxicosis.

KEY WORDS: ***Acute bovine liver disease, cattle, hepatic, Jamesdicksonia dactylidis, mycotoxicosis, photosensitisation***

Introduction

Photosensitivity results when a photodynamic agent causes the skin (particularly areas without hair or pigmentation) to become sensitised to light of certain wavelengths (Smith and O'Hara 1978). The most prevalent type of photosensitisation is hepatogenous, where phytoporphyrin (phylloerythrin), a photodynamic derivative of the green plant pigment chlorophyll, accumulates in the systemic circulation due to liver damage that results in the impairment of the normal biliary excretion of this compound. Photosensitivity can be a clinical sign in facial eczema, acute bovine liver disease (ABLD), microcystin-induced hepatic disease caused by the consumption of water containing certain cyanobacteria, lupinosis due to a mycotoxin from the fungus *Phomopsis leptostromiformis*, as well as poisoning by a number of hepatotoxic plants, such as *Lantana camara* (Cheeke and Shull 1985; Smith and Towers 2002; Lancaster *et al.* 2006). In some instances, the cause of outbreaks of photosensitivity in grazing livestock is unknown (Campbell *et al.* 2010). The major differential diagnoses of photosensitisation in cattle in Tasmania, Australia, are facial eczema, ABLD, or mycotoxicosis of unknown cause.

Facial eczema is caused by sporidesmin, a hepatoxic mycotoxin produced by spores of the saprophytic fungus, *Pithomyces chartarum* in warm, humid weather (Smith and Towers 2002). The first clinical signs of facial eczema are usually transient diarrhoea, inappetence, and a sudden pronounced drop in milk production followed by photosensitivity 7–20 days after exposure to pastures with high *P. chartarum* spore counts (Smith and Towers 2002; Di Menna *et al.* 2009).

Acute bovine liver disease, previously known as phytotoxic hepatitis, is a syndrome of acute liver failure followed by photodynamic dermatitis (Lancaster *et al.* 2006). The cause of ABLD is unknown and there are limited descriptions of the disorder in peer reviewed scientific literature. It has been reported in beef and dairy cattle in mainland southern Australia and Tasmania (Lancaster *et al.* 2006). The first signs of illness reportedly

ABLD	Acute bovine liver disease
DON	Deoxynivalenol

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http://dx.doi.org/10.1080/00480169.2016.1232181

occur within 12–24 hours of cattle being introduced to spelled pastures. Outbreaks appear to be associated with recent rain, autumn, the presence of rough dog's tail (*Cynosurus echinatus*) and calm, warm, sunny weather with heavy morning dews (Lancaster *et al.* 2006). The epidemiology of ABLD suggests a mycotoxicosis, but there are no known mycotoxins, with the exception of amatoxins from some mushroom species, that produce hepatic periportal necrosis (Lancaster *et al.* 2006).

Mycotoxicoses in general, such as facial eczema, are caused by toxins released from fungi and are distinct from mycoses, diseases caused by fungal growth, not fungal toxins (Galey 1996). Mycotoxicoses of grazing animals, other than facial eczema, include fescue toxicosis, lupinosis, paspalum staggers, ryegrass staggers, zearalenone infertility, and slaframine toxicosis (Smith and Towers 2002; Bryden 2012). Other well-known mycotoxins that affect livestock are aflatoxins and ochratoxin A (mainly produced in storage conditions by *Aspergillus* and *Penicillium* fungal spp.), trichothecenes and fumonisins (both produced by *Fusarium* spp. in cereals), and ergot alkaloids (Obremski *et al.* 2009). Concurrent exposure to multiple mycotoxins often occurs in livestock (Zaki *et al.* 2012), and this exposure creates variable clinical signs and pathology that are not pathognomonic for any particular toxin.

Mycotoxicoses conform to a few general principles, including the cause may not be immediately identified, they are not transmissible from one animal to another, treatment with therapeutic agents or antibiotics has little effect on the course of the disease, outbreaks are often seasonal, because particular climatic sequences may favour fungal growth and toxin production, epidemiological studies indicate a specific association with a particular feed, and examination of feedstuffs may reveal the presence of usually large numbers of fungi; however, this does not necessarily indicate that mycotoxins have been produced (Schiefer 1990).

Here we describe a large outbreak of acute photosensitisation and mortality of unknown cause, but differing from ABLD, in a herd of dairy cattle in Tasmania.

Case history

The case herd was on a dairy farm located at Gretna in the Derwent Valley in Tasmania, Australia. In the 1980s, the same farm had an outbreak of undiagnosed photosensitisation and mortalities, and staggers associated with the ingestion of phalaris (*Phalaris aquatica*) was believed to be the cause of death of approximately 50 heifers in the late 2000s. Since 2012 heifer growth was reported to be "very poor". On 3 October 2014 (Day 0) during a morning milking, the farm owner was alerted by the automated milk recording system to 94 of the 678 cows milked that day that had abnormally low production.

The herd was predominately spring-calving and consisted of Holstein, Jersey, and Holstein cross Jersey cattle of mixed ages that were a mean of 77 (SD 116) days in milk. The 7-day mean milk yield and bodyweight were 25.3 (SD 8.7) litres and 436 (SD 87) kg, respectively, based on 2 days prior for cows >10 days in milk. The mean, minimum and maximum ambient temperature at 15:00 for the 14 days before Day 0 were 17.3 (SD 3.7), 5.7 (SD 3.5), and 19.2 (SD 3.7)°C, respectively. The mean temperature humidity index at 15:00, maximum wind speed, and rainfall for the 14 days before Day 0 were 60.2 (SD 3.6), 50.2 (SD 26.7) km/hour, and 0.5 (SD 0.9) mm, respectively (Anonymous 2014).

The water source was the large, rapidly flowing Styx River. The herd had been grazed on a rotation of irrigated, predominately perennial ryegrass (*Lolium perenne*) at night and dryland pasture during the day. The herd was fed wheat according to production in the parlour twice daily with high producing cows receiving up to 8 kg/day of wheat inclusive of 400 g of canola meal and 250 g of RT250 (monensin, macro and trace minerals; Cows-R-Us, Camden, NSW, Australia). A new load of wheat had been delivered 8 days earlier. Farm staff reported there was a lot of residual pasture remaining on the dryland area of pasture (5 ha) offered to the cows 2 days earlier. The herd was subsequently re-grazed on that dryland pasture the following day. It was the first time this dryland pasture had been grazed in approximately 3 months, as pasture growth was slow due to lack of rain. Approximately 300 cows from the milking herd had returned to the dryland break after 06:00 but were removed from the pasture by 08:00 following advice from the farm's consultants and veterinarian.

Clinical findings

On Day 0, the condition of the herd was extremely serious. A range of clinical signs was evident that included a sudden drop in milk production, recumbency, peracute photosensitisation, inflammation of the coronet, conjunctival erythema, distress indicated by kicking at the flank, bruxism, discomfort, weight shifting, vocalisation indicating pain, depression, low hung heads, and hunching, but no cows were dead. A minimum of 165 cows of the 678 cows in the herd were identified as clinically affected (morbidity 24.3%) and were treated with20 mL I/M ketoprofen (100 mg/mL; Ilium Veterinary Laboratories, Smithfield, NSW, Australia) daily for up to three consecutive days. Only cows that were severely affected were given a further treatment. Treatment began during the afternoon milking of Day 0, but not all clinical cases received a treatment on this day. On Day 1 three cows died. Ancillary treatments included use of shaded pasture for affected cows and use of udder cream containing boric acid and zinc oxide (Ruddock's DeriSal; Sykes Vet International Pty. Ltd., Dandenong South, VIC, Australia).

By Day 4 a total of seven cows had died, and severely affected cows had been separated into a sick herd that was provided with shade, minimal walking, and continued treatment. These animals presented with low rumen fill, drooling, dehydration, serous ocular discharge, bruxism, periauricular and periorbital oedema resulting in drooped ears and closed eyes, and submandibular oedema Some had severe signs of pain, including hunched backs, grunting, kicking at the flank and kicking out, severe inflammation and, in some cases epithelial erosion of mucous membranes of the nares, and severe erythema of the udder.

On Day 5, 38 cows were severely affected, 39 were recovering, and 13 were "doubtful" within the sick herd. On Day 6, cows that were not fit to be milked were separated from the remaining sick herd. On Day 7, a large number of cows had mastitis associated with severe ulceration of udder skin and teat ends and were treated. On Day 11, cows had varied presentation of clinical signs. Large sheets of skin were sloughing off the udders, scabs had formed, and some cows had skin sloughed off their body and ears and erythema of the teats. Some irritation was occurring during milking. By Day 12 a total of 29 cows were being treated for mastitis.

Mortality over approximately the first 30 days was 19/678 (2.8%) milking cows.

Six weeks after the onset cows that were dried off as a result of the illness were in good body condition, 3.5 on the 1–5 Edmondson scale (Edmondson *et al*. 1989) and had some signs of healing skin on their body and udder. Cows in the lactating herd were in different stages of healing. Scabs and sloughing were predominately restricted to hairless or non-pigmented regions. Cows did not appear distressed or in pain and were in fair condition (score 3).

In the months subsequent to the outbreak, re-opening of wounds following pruritus occurred for some cattle. Some cows did not show sufficient recovery from the disease and were culled as a direct result of the disease or due to secondary factors that may have been a result of the disease, e.g. poor fertility, mastitis, or low production.

At examination of the herd 9 months after the start of the outbreak no signs of scar tissue were evident on udders. Signs of healed skin were evident for unpigmented skin on the bodies of some cattle. An unknown, but large, percentage of the affected cows recovered and remained in the herd. Skin sloughing, suggestive of a mild photosensitisation was evident in a small number of young stock approximately 12 months after the original case. No mortalities were reported and it is unknown whether this was the same condition.

Clinical chemistry

Blood samples were collected in plain and lithium heparin-coated tubes on Day 4 from five clinically normal and five clinically affected cows, and analysed for liver and muscle enzyme activities, blood biochemistry and haematology by the Animal Health Laboratory (Tasmanian Department of Primary Industries, Parks, Water, and Environment (DPIPWE), Kings Meadow, Tasmania, Australia). Results for the two groups were compared using a median test using Statistix 10 (Analytical Software, Tallahassee, FL, USA). Results for the two groups are reported in Table 1, along with reference ranges. Compared with the reference ranges, activities of aspartate aminotransferase, glutamate dehydrogenase, and gamma-glutamyl transferase were raised in the clinically affected cattle. Although within the reference range, activity of creatine kinase, as well as concentrations of total bilirubin, sodium, and chloride were higher, and concentrations of magnesium lower, in the clinically affected than the clinically normal cows.

The pathologist's comments were that all samples from clinically affected cows suggested they had cholangiohepatopathy and some had cholestasis. Muscle injury was possible in two cows and hyperglobulinaemia, consistent with inflammation, was present in two cows. Low creatinine, consistent with weight loss and loss of muscle mass was evident in both clinically affected than the clinically normal cows. Some clinically normal cows had marginal changes that suggested mild dehydration and mild subclinical hepatopathy.

Pathological findings

Necropsies carried out on two cows selected for euthanasia on Day 4 showed distension of the gall bladder, excess peritoneal fluid (in one cow), profound swelling of the liver and marked and extensive icterus. Both cows were in poor condition with very little abdominal fat. A further two cows were necropsied on Day 5. One cow was ataxic, mostly recumbent, could stand,

Table 1. Median, minimum, and maximum values for clinical chemistry results for blood samples collected from cows without (normal; n=5) and with clinical (affected; n=5) signs 5 days after cows were observed with sudden-onset photosensitisation in a dairy herd in Tasmania, Australia

	Clinically normal[a]		Clinically affected			
Measure	Median	Min, max	Median	Min, max	P-value[b]	Reference range[c]
Creatine kinase (U/L at 37°C)	129	98, 180	278	242, 363	0.008	0–280
Aspartate aminotransferase (U/L at 37°C)	83	69, 109	269	190, 564	0.008	0–130
Glutamate dehydrogenase (U/L at 37°C)	14	9, 28	68	43, 199	0.008	0–21
Gamma-glutamyl transferase (U/L at 37 °C)	22	19, 26	185	133, 269	0.008	0–42
Total bilirubin (μmol/L)	2.6	2.3, 2.6	5.5	4.4, 101.9	0.008	1.7–8.1
Creatinine (μmol/L)	54	42, 66	51	40, 60	1.0	85–178
Urea (mmol/L)	2.9	2.1, 4.4	2.9	2.8, 3.8	1.0	2.1–10.7
Calcium (mmol/L)	2.3	2.2, 2.6	2.3	2.1, 2.4	0.486	2.1–2.8
Magnesium (mmol/L)	1.01	0.97, 1.05	0.9	0.7, 0.92	0.008	0.7–1.2
Phosphate (mmol/L)	1.59	1.32, 1.9	1.63	1.11, 1.86	1.0	1.2–2.5
Sodium (mmol/L)	136	135, 138	139	138, 140	0.029	132–152
Potassium (mmol/L)	4.7	4.3, 5.1	4.7	4.2, 5.4	1.0	3.9–5.8
Chloride (mmol/L)	99	98, 102	103	103, 107	0.008	97–111
Protein (g/L)	70	67, 71	68	58, 77	1.0	60.0–85.0
Albumin (g/L)	37.3	31.9, 39.1	33.8	28.3, 35.8	0.107	24–38
Globulin (g/L)	32.2	21.8, 35.1	34.3	29.5, 40.8	1.0	30.0–34.8
Albumin/globulin ratio	1.16	0.91, 1.22	0.96	0.88, 1.15	0.107	0.63–1.20
β-hydroxybutyrate (mmol/L)	0.35	0.27, 0.48	0.43	0.33, 0.59	1.0	0.0–0.8
Packed cell volume	31	27, 35	30	29, 36	1.0	24–46

[a] Cows that were not showing any clinical signs at the time of sampling.
[b] Significance of Fisher's exact test.
[c] From Central Veterinary Diagnostic Laboratory (CVDL), 1997.

was shivering, and had severe photosensitisation, and was subject to euthanasia. The liver was enlarged and severe hepatic damage was evident as indicated by variable discolouration. Oedema was present around the distal abomasum with excoriation of multiple strips of mucosa. The second cow was dead and bloated, but had been dead <12 hours. Peritonitis was evident and the liver was enlarged with an orange cut surface. Ulceration of the abomasal mucosa was present and the abomasal wall was ruptured at one of the ulcerated sites.

Tissues were fixed in 10% neutral buffered formalin for 24 hours, processed overnight in a tissue processor (TP1050 Leica Microsystems, Wetzlar, Germany). Paraffin embedded tissues were then cut into 3 µm sections and stained with H&E. Liver samples collected on Day 4 and 5 showed mild to moderate periacinar necrosis of hepatocytes (Figure 1) with expanded periacinar sinusoids and mild mixed inflammatory infiltrate; there was also very mild periportal biliary hyperplasia and rare neutrophils, macrophages, and lymphocytes around the periportal areas; one liver sample had mild infiltrates of lymphocytes, plasma cells, and eosinophils within periportal regions, and increased numbers of bile ducts. Rare bile ducts were dilated and had attenuated epithelium. Periportal hepatocytes had increased lightly eosinophilic and feathery cytoplasm indicating glycogen. Small numbers of Kupffer cells contained a gold-coloured cytoplasmic pigment. There were increased numbers of bile ducts and inflammatory cells within the periportal regions. The pathologist noted that the findings suggested a hepatic insult which could have been caused by a range of toxins, consistent with diagnoses of cholangio-hepatitis and were not consistent with histopathology described for ABLD. The lesions in the livers collected on Day 5 reflected chronic inflammatory changes and were also considered not typical of ABLD.

Abomasal ulceration was identified in the two cows necropsied on Day 5, one of which had a ruptured ulcer that lead to peritonitis and the other of which was diagnosed with gastritis that was ulcerative, sub-acute, focally extensive, and severe.

Faecal samples collected on Day 5 were examined at the Animal Health Laboratory, (DPIPWE, Kings Meadow, Tasmania, Australia). No acid-fast bacilli were detected when examined using a Ziehl–Neelsen stain and there was no evidence of Johne's disease using microscopy (Gwozdz 2010). Faecal cultures showed no significant growth and no *Salmonella* spp. or *Yersinia* spp. were isolated. No ova from liver fluke, *Strongylid* spp., or *Nematodirus* spp. were detected.

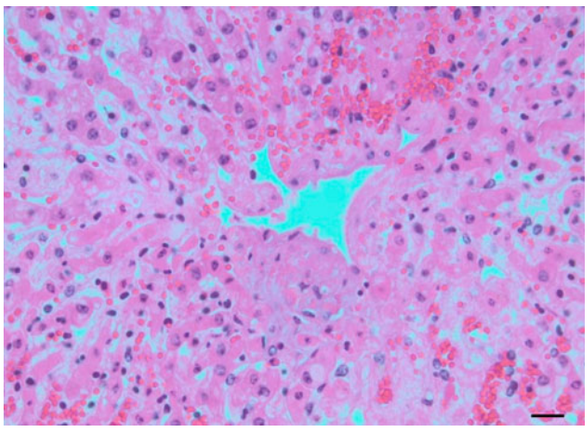

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Figure 1. Photomicrograph of a section of liver from a cow that died 4 days after cows were observed with sudden-onset photosensitisation in a dairy herd in Tasmania, Australia. Mild to moderate periacinar necrosis of hepatocytes can be seen (H&E, bar=20 µm)

Mycology and mycotoxicology

Feed

The wheat the herd was fed was considered to be an unlikely cause of the illness and was unlikely to contain high levels of mycotoxins based on visual assessment. No signs of contamination or discolouration were evident in the wheat. No samples were submitted for further analysis.

The dryland pasture was predominately barley grass (*Hordeum murinum*) and phalaris, and over sown with annual (*L. multiflorum*) and perennial ryegrass, and contained traces of subterranean clover (*Trifolium subterraneum*) and winter grass (*Poa annua*). The pasture break was on a westerly facing slope and had been grazed to a residual length of 2–3 cm. Residues of summer grasses were present in the under storey. Rough dog's tail was occasionally present in the under storey. An unusual black spot fungus was observed in the senescent leaves of barley grass in the deeper sward that was extending in places into the live leaves and also on some unidentified dead plant litter from summer at the base of the sward (Figure 2). This fungus was prominent across the middle of the paddock that the cows had grazed, but was not observed in the adjacent paddock. Areas of plant tissue adjacent to the black fungus were pale or yellow.

Mycology

Two samples of dryland pasture collected on Day 5 both had total fungal spore counts conducted at the Plant Breeding Institute (University of Sydney, Cobbitty, NSW, Australia) using the method of Oldman and Di Menna (1983). Total spore counts were 115,000 spores/g of leaves, with *Alternaria* spp. spores identified at 20,000 and 25,000 spores/g of leaves in the two samples, respectively. No *P. chartarum* spores were reported.

Colour online, B/W in print

Figure 2. Photograph of a black spot fungus on senescing barley grass (*Hordeum murinum*) leaf found in the paddock grazed by cows before the sudden onset of *f* photosensitisation in a dairy herd in Tasmania, Australia. Note pale or yellow areas of plant tissue adjacent to the black fungus

A mature barley grass plant absent of seed heads that had black spots on the leaves, was collected on Day 12 from the dryland pasture the herd had been grazing prior to the onset of clinical signs. A leaf smut, *Jamesdicksonia dactylidis* was identified, based on spore morphology. The sample of barley grass had been stored for several weeks in a refrigerator and was contaminated with soil. Endophytic fungi were isolated from a random selection of leaves and stems by the method of Wong *et al.* (2015). The pure cultures were incubated at 20°C and 25°C in the dark, and at room temperature (20–25°C) in diffuse light on a laboratory bench for a further 4 months to induce sporulation in cultures that had not sporulated. Four fungi were consistently isolated from the leaves. Two fungi sporulated in culture and one fungus was identified as *Nigrospora* spp. The other was an ascomycete, producing numerous black ascomata in culture, but has not yet been identified. The other two fungi have not sporulated in culture.

Mycotoxicology

A sample of barley grass that was collected on Day 12 was freeze-dried and analysed at the Interuniversity Department for Agrobiotechnology (IFA Tulln, University of Natural Resources and Life Sciences, Vienna, Austria). The method was a high-performance liquid chromatography-electrospray ionization-mass spectrometry (HPLC-ESI-MS/MS) based multi-mycotoxin method. using an Agilent 1290 HPLC (Agilent Technologies, Santa Clara, CA, USA) coupled to an Applied Biosystems 5500 QTrap mass spectrometer (Waltham, MA, USA). The results were interpreted by BIOMIN Holding GmbH (Getzersdorf, Austria). From this sample, 55 mycotoxins were identified; a detailed list of mycotoxins and other metabolites detected, and their concentrations, is provided in Supplementary Table 1.[1] B-trichothecenes (3,935 µg/kg), fumonisins (8,081 µg/kg), and zearalenone-metabolites (1,375 µg/kg) were detected at concentrations presenting an extremely high risk of biological harm to cattle. Concentrations are considered high when they are >1,000, >4,000, and >250 µg/kg, respectively. Concentrations of ergot alkaloids presented a medium risk (136 µg/kg) and A-trichothecenes (8.67 µg/kg) a low risk. A medium risk of ergot alkaloids is considered within 100–400 µg/kg and a low risk of A-trichothecenes is considered at <100 µg/kg. Aflatoxin B1, ochratoxin A, and aflatoxins were not detected.

Prevention

The dryland pasture suspected of causing the condition was sprayed out with 2 L/ha of glyphosate and was reseeded. Fungicides and burning the paddock were considered but not utilised. A mycotoxin binder (Biofix Plus, BIOMIN Holding GmbH) which contains yeast cell walls, natural microbials, and bentonite is now added to the lactating cow ration and no further cases have been reported in the lactating herd up until May 2016.

Discussion

We have described the investigation of an outbreak of acute photosensitisation and mortality of unknown cause. The key findings were the prominent clinical sign of photosensitisation, the rapid onset of the disorder, mortality, high morbidity, milk drop, elevated liver enzymes, and presence of epithelial erosions. Our morbidity estimate is likely to be conservative as a number of cows were probably subclinical when first assessed. The main differential diagnoses were facial eczema, ABLD, or an undescribed mycotoxicosis. Lantana and lupins were not present in the paddock and the water source was fresh and flowing; therefore lantana poisoning, lupinosis, and microcystin poisoning resulting from cyanobacteria were dismissed as causes of photosensitisation.

Facial eczema was considered a likely differential diagnosis. However, the rapidity and acute onset of clinical signs in this case, absence of *P. chartarum* spores, and inconsistent histopathology, namely absence of periportal necrosis and occurrence of epithelial erosions, do not support the diagnosis of facial eczema. In addition, environmental conditions were not conducive to *P. chartarum* growth, but no spore counts were conducted in the weeks prior to clinical onset so its presence can not be eliminated.

Another probable differential diagnosis was ABLD. In Tasmania most cases of ABLD have been observed in the Northern Midlands and South, particularly in the Derwent Valley and Copping areas (Anonymous 2015). There is limited published information on ABLD, the aetiology is unknown, and it is not clear if there is a definitive pathophysiology. Lancaster *et al.* (2006) produced a description of ABLD in Australia based on 15 clinical reports which supports the belief that periportal necrosis occurs. Findings from this case that supported the differential diagnosis of ABLD, based on the description by Lancaster *et al.* (2006), include the first signs of illness occurred with 12–14 hours of cattle being introduced to a spelled pasture, the pasture had a range of improved pasture species with considerable decaying residue from previous growth, dry rough dog's tail was present (although rare), signs of photodynamic dermatitis occurred a few days after initial clinical signs and was of a severity that required humane slaughter, other causes of liver damage such as blue-green algae poisoning were eliminated, liver enzymes were elevated suggestive of liver damage, and a drop in milk production occurred. The epidemiology and histopathology in this case are not consistent with the limited knowledge of ABLD described previously. Periportal necrosis was not consistent with damage that is fatal. The pathologist commented that the mild to moderate necrosis was insufficient to cause overwhelming liver failure as >60% of the liver parenchyma was unaffected. The outbreak occurred in October and pasture growth conditions were very slow with conditions in the previous fortnight being dry and windy which is not consistent with previous outbreaks that appeared to be associated with autumn and calm, warm, sunny weather conditions with heavy dews that favoured rapid pasture growth (Lancaster *et al.* 2006).

Attention has focussed on the combination of rough dog's tail and *Drechslera* spp. (saprobic fungi) as causal agents of ABLD as these have been consistently associated with the syndrome (Lancaster *et al.* 2006). The grass itself is unlikely to be toxic (Lancaster *et al.* 2006), but extracts of *C. echinatus* collected from toxic pastures at the time of an ABLD outbreak have been shown to be hepatocytotoxic (Aslani *et al.* 2006). However, in a feeding study rough dog's tail and *D. biseptata* did not cause ABLD, although the grass used was collected at a time of year that ABLD does not occur, fungal culture conditions were very different to those in the field reports, and only 9.5% of the oat grains fed in the experiment were infected with *D. biseptata* (Lancaster

[1] http://dx.doi.org/10.1080/00480169.2016.1232181

et al. 2006). In our case, the involvement of rough dog's tail and *Drechslera* spp. cannot be ruled out as samples of rough dog's tail were not examined for mycology.

We consider that the most likely diagnosis is an undescribed mycotoxicosis. This is the first record of *J. dactylidis* in Tasmania, and on barley grass. There are records of *J. dactylidis* from Victoria on rough dog's tail and from NSW on Cocksfoot (*Dactylis glomerata*; Vánky and Shivas 2008) and also on some species in New Zealand (McKenzie and Vánky 2001). However, *J. dactylidis* has not been shown to be capable of producing toxigenic mycotoxins. Several other fungal endophytes were isolated from the grass, but as they have not been identified to species level, no conclusions on their role in possible mycotoxicosis can be drawn.

The biological impacts of many of the mycotoxins detected from the barley grass sample are unknown, as are their possible interactions which could be additive, synergistic, or antagonistic. High concentrations of mycotoxins or multiple mycotoxins limit the ability of rumen microflora to detoxify them, allowing them to pass into the duodenum where they are absorbed, potentially causing damage to organs (Kiessling *et al.* 1984). Large numbers of mycotoxins and multi-toxin occurrences can create a divergence of observed clinical effect in field outbreaks, and pose a challenge for diagnosis (Zaki *et al.* 2012). In the present case signs of mild photosensitisation were reported to be higher than in herds nearby the case herd, suggesting the presence of mycotoxins in the area. It should be noted that optimal conditions for fungal growth are not necessarily optimal for toxin production (Zain 2011).

Diagnosis in cases of mycotoxicosis are likely to be difficult and rarely definitive. These cases pose a challenge because lesions may be non-specific, for example hepatic necrosis can be caused by many toxins. Additionally, effects of mycotoxins may be masked by secondary effects, necropsy autolysis may prevent observations of subtle lesions, lesions may occur too late for a causal relationship to be confidently established, interactions of mycotoxins may produce unusual lesions that are misdiagnosed, the causal agent may no-longer be present at the time of investigation, and extrapolation of findings between species is difficult (Schiefer 1990).

The well-known mycotoxins, trichothecenes, zearalenone, and fumonisins were detected in extremely high concentrations in the barley grass sample analysed. Trichothecenes are divided into types A and B and are often present in combination with zearalenone (Richard 2007), as occurred in this case. They are mainly produced by fungi from the *Fusarium* genus (Eriksen and Pettersson 2004). The most common A-trichothecene is T-2 toxin (low concentrations in this case) and the most common Type B is DON (high concentration in this case). The tolerance of dairy cattle to DON is controversial as it can be metabolised to a less toxic de-epoxide metabolite by rumen microflora (Fink-Gremmels 2008). The most important mode of action of trichothecenes is as inhibitors of protein synthesis which can be followed by secondary disruption of DNA and RNA synthesis. Actively dividing cells are affected which can lead to severe gastroenteritis, skin necrosis, immune impairment, and initiation of shock (Richard 2007). Inflammation of the nose, lips, mouth, and tongue is common, and ulcers can occur (Wyllie and Morehouse 1978).

Fumonisins are not degraded in the rumen and therefore pose a high risk to ruminants. Fumonisins are produced by *Fusarium moniliforme* and are hepatotoxic, neurotoxic, and carcinogenic mycotoxins (Galey 1996). They interfere with protein synthesis and sphingolipids, which are found in the brain and liver and are vital for cell growth, differentiation, and transformation (Wang *et al.* 1991; Galey 1996).

Opportunities to undertake extensive examination and retrospective analysis of large outbreaks of disease are often limited by resources. In this case considerable resources were applied to the investigation. However, diagnosis in this case could have been aided by more necropsies, biopsies, histopathology, and additional blood analyses. Further pasture sampling for mycology and mycotoxicology would also have been beneficial, particularly from the senescent deep litter that cattle may have inadvertently grazed. Unfortunately, such outbreaks are sporadic therefore efficient avenues of analysis are not always available for clinicians, and comprehensive diagnostics can be expensive. Further molecular evaluation of the fungal cultures is being considered.

In conclusion, a definitive diagnosis could not be reached in this case of acute photosensitisation and mortality in dairy cattle grazing possibly contaminated dryland pasture. The combination of clinical signs, blood analyses, pathology, histopathology and epidemiology differed from both facial eczema and ABLD, suggesting an undescribed mycotoxicosis that was associated with the presence of a black fungus and very high mycotoxin concentrations in a small sample of pasture.

Acknowledgements

The authors wish to thank taxonomist Dr. Roger Shivas (Plant Pathology Herbarium – Biosecurity Queensland, Department of Agriculture and Fisheries, Brisbane, QLD, Australia) for identification of *J. dactylidis* and BIOMIN Holding GmbH for financial support for the mycotoxin binder and mycotoxin analysis. The authors also wish to acknowledge the veterinary pathologists, Mr. A. Davis and Mr. Graeme Knowles and staff (Animal Health Laboratory, Tasmanian Department of Primary Industries, Parks, Water, and Environment, Kings Meadow, TAS, Australia) for their involvement in the case, Ms. Rebecca Atkinson for fungal spore counting (Animal Health Laboratory, Tasmanian Department of Primary Industries, Parks, Water, and Environment), The Dairy Science Group (Faculty of Veterinary Science, The University of Sydney, Camden, NSW, Australia) for the use of their centrifuge, and Dr. P. Dracatos and Dr. W. Cuddy for examining the barley grass sample (The University of Sydney, The Plant Breeding Institute, Cobbitty, NSW, Australia). The attitude and persistence of the farm staff during the outbreak should be commended.

This work was supported by the Biomin; The University of Sydney; Tasmanian Department of Primary Industries, Parks, Water, and Environment; Scibus.

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Submitted 23 October 2015

Accepted for publication 10 August 2016

First published online xx xxxx xxxx

*Non-peer-reviewed